ABSTRACT

Ph.D. Rajadurai Rajamahendran Animal Science (Reproductive Physiology)

ROLE OF OVARIAN STEROIDS ON ESTRUS CONTROL IN BOVINE

A simple and inexpensive vaginal device consisting of silastic tubing containing progesterone and estradiol-17β was developed to control estrus in cycling heifers. More than 90% of the heifers retained the device and there was precise synchronization of estrus after removal.

Effects of silastic vaginal device containing either estradiol-17β, progesterone or progesterone + estradiol-17β on estrus and LH release were studied in six ovariectomized heifers. Results showed that progesterone blocked estrogen induced estrus and LH release.

Influence of progesterone priming on induction of puberty was studied in 32 heifers. The results demonstrated that progesterone pretreatment increased the response to an ovulatory stimulus.

Effects of silastic vaginal devices containing either progesterone, progesterone + estradiol-17β or no steroid on progesterone and estrogen cytosol receptors in target organs were studied in 11 prepuberal heifers. A significant decrease in uterine estrogen receptors occurred in steroid treated heifers. Specific progesterone and estrogen receptors were demonstrated in anterior pituitary and hypothalamus but no difference was observed in receptor concentrations.

RESUME

Ph.D. Rajadurai Rajamahendran Zootechnie (Physiologie de la Reproduction)

ROLE DES STEROIDES OVARIENS SUR LE CONTROLE DE L'OESTRUS CHEZ LES BOVINS

Le contrôle de l'oestrus chez les génisses cycliques a été évalué grâce à un nouveau dispositif intravaginal simple et non dispendieux, fait de tube silastique contenant de la progestérone et de l'oestradiol-17β. Ce dispositif a été retenu par plus de 90% des sujets et, après son retrait, la synchronization de l'oestrus s'est avérée précise.

Les effets de ce dispositif contenant soit de l'oestradiol-17β, de la progestérone ou en combinaison sur l'oestrus et la secrétion de LH ont été étudiés chez six génisses ovariectomisées. Comme les résultats le montrent, la progestérone a bloqué l'oestrus induite par l'estrogène et la secrétion de LH.

L'influence de la progestérone sur l'initiation de la puberté a été analysée chez 32 génisses. Les résultats démontrent l'augmentation de la réponse à un signal ovulatoire suivant un prétraitement avec de la progestérone.

Les effets du dispositif intravaginal soit vide ou avec de la progestérone, ou de la progestérone et d'oestradiol-17β sur les récepteurs cytoplasmiques des organes cibles pour l'estrogène et la progestérone ont été évalués chez 11 génisses prépubères. Une diminution significative dans les récepteurs estrogéniques utérins s'est manifestée chez les sujets traités avec les stéroides. Des récepteurs spécifiques pour la progestérone et l'estrogène ont été trouvés dans l'adénohypophyse et l'hypothalamus. Cependant, aucune différence n'a pu être établie dans le nombre de ces récepteurs.

ROLE OF OVARIAN STEROIDS ON ESTRUS CONTROL IN BOVINE

by

Rajadurai Rajamahendran

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Department of Animal Science,
Macdonald Campus of McGill University,
Montreal, Quebec, Canada.

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Suggested short title -

ESTRUS CONTROL IN BOVINE

RAJAH

DEDICATED TO MY MOTHER

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CURRICULUM VITAE

Name -	Rajadurai Rajamahendran
Permanent Address -	"Mahes Vasa", Navaly South, Manipay, Sri-Lanka.
Date of Birth -	July 19, 1944
Place of Birth -	Jaffna, Sri-Lanka
Marital Status -	Married - two children

Formal Education

University of Ceylon, Sri-Lanka
B.V.Sc. October, 1968

McGill University, Montreal, Quebec
M.Sc. November 1974

Experience

March 1969 to October 1970	Government Veterinary Surgeon, Department of Agriculture, Sri-Lanka
November 1970 to August 1972	Assistant Lecturer in Applied Animal Physiology, Faculty of Agriculture, University of Ceylon, Sri-Lanka.
September 1972 to present	Graduate student, Department of Animal Science, McGill University. Colombo Plan Scholar - on study leave from the University of Ceylon, Sri-Lanka.
Professional Societies	Society for the Study of Reproduction American Society of Animal Science Canadian Society of Animal Science

Papers Published

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INTRODUCTION

The development of artificial methods for regulating the estrous cycle in various species of farm animals has long been a goal of animal physiologists. This regulation of estrous cycle or synchronization causes many females to exhibit estrus on the same day or within a period of two to three days. Effective methods for controlling the time of estrus and ovulation in bovine would have several practical and experimental applications.

Estrus control in bovine will likely find its greatest application in the commercial beef operation. The difficult task of heat detection while cows are on pasture has practically prevented the use of artificial insemination. However, estrous synchronization in large groups of cows will result in more widespread application of artificial insemination in these operations. In the dairy industry estrus control will probably be used mainly to breed a group of heifers on a single day. This will enable more heifers to be bred artificially and will reduce the number bred naturally, while on pasture, by bulls of questionable genetic merit. Increasing the size and efficiency of dairy operation has encouraged the practice of raising large numbers of replacement heifers as a specialized operation. Development of successful methods for synchronizing

estrus is expected to accelerate this trend, since it will make it possible to market groups of heifers bred to calve on given dates.

Another important reason for synchronizing estrus in bovine would be to reduce the spread of parturition dates, especially when followed by methods designed for the induction of parturition. This would reduce the labor required for calving, and would increase the efficiency through the feeding and handling of uniform groups of growing and finishing animals. Specialized raising and feeding areas could aid in the prevention and control of disease, especially in young calves.

One of the most important applications of estrous synchronization in the bovine is to increase the efficiency of embryo transfer. A close synchronization of the estrous cycles of the donor and the recipient animal is essential to obtain successful pregnancy following transfer.

Many procedures are available to synchronize estrus in cattle. Removal of the corpus luteum by manipulation of the ovary through the rectal wall results in estrus and ovulation. This method is not used routinely because of the high incidence of adhesions around the oviduct and excessive haemorrhage after removal of the corpus luteum. The other procedure involved the use of progestins to prevent estrus and ovulation until all corpora lutea have regressed. Progestins have been administered

in the feed, drinking water, as subcutaneous and vaginal tampons. Best synchronization results have been obtained by treating with human chorionic gonadotrophin, estradiol benzoate or gonadotrophin releasing hormone after the termination of progestin treatment. However, the conception rate following the synchronized estrus are 10 to 15% below that of control animals.

Recently it has been reported that use of short-term progesterone + estrogen combination treatment to synchronize estrus in bovine has improved the fertility rates. Further work is necessary in this regard to develop a satisfactory treatment regimen involving progesterone and estrogen to synchronize estrus in bovine and become practical to the livestock producer. Furthermore, the role of progesterone in the control of estrus and ovulation in bovine is yet to be identified.

The other major approach to estrus synchronization is to cause rapid luteal regression by prostaglandins, particularly prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Practical application of $PGF_{2\alpha}$ to synchronize the estrous cycle in domestic animals has been attempted in many laboratories. Studies in cattle indicate that administration of $PGF_{2\alpha}$ between day 5 and 16 of the cycle causes most of the animals to come into estrus in about 72 hours post-treatment. Fertility following treatment is reported to be normal. Double injections of prostaglandins $F_{2\alpha}$ given

11 or 12 days apart were able to synchronize all heifers, including those between day 0 and 4 of the cycle. In spite of the encouraging synchronization results, $PGF_{2\alpha}$ has known side effects and hence it may not be the drug of choice for estrus synchronization in cattle.

Puberty is an important reproductive phenomenon in dairy and beef heifers. With increased emphasis on breeding heifers to calve at two years of age, extreme delays in reaching puberty will have a detrimental effect on breeding efficiency. There is increasing evidence that heifers bred to calve just at two years of age produced more calves in their life time than those calving first at three years or older. To maximize efficiency and profit, heifers must reach puberty early and be cycling during the breeding season, hence induction of puberty by hormonal manipulation is indicated.

Induction of puberty in prepuberal heifers will find its greatest application in ova transfer. It has been shown that prepuberal calves respond to superovulation treatment better than cycling and post partum cows; however, the recovery and fertility rate seems to be low. This has been attributed to poor reproductive tract development. Therefore, by inducing puberty in superior females using hormonal treatment, it may be possible to obtain a much greater number of offspring by ova transfer. Furthermore, progeny testing of females could

be achieved at a much earlier age.

Many attempts have been made to develop a satisfactory treatment, using different combinations of steroid and gonadotrophic hormones to induce puberty in prepuberal heifers. To date, short-term progestagen implant treatment coupled with an injection of estrogen has given the best results. Further work is necessary to develop a simple, reliable and satisfactory hormonal treatment to induce cyclic activity in prepuberal heifers.

In the field of research, the hormonal induction of puberty offers a very useful tool to study the hormones involved in the onset of natural puberty. Studies on induction of puberty and hormonal concentrations at onset of natural puberty strongly suggest that progesterone is involved in the final events leading to puberty in bovine. The mechanism of its action is yet to be determined.

LITERATURE REVIEW

Bovine females first exhibit estrus at 8-18 months of age when they weigh about 250 kg. There is a wide range in age and weight at puberty and this range depends on breed and level of nutrition (Morrow, 1969). Estrus occurs at fairly regular intervals usually within a range of 18-24 days. The interval from the beginning of one estrus to the next is called the estrous cycle. The estrous cycle can be divided into four phases called proestrus, estrus, metestrus and diestrus. Proestrus is characterized by increase in follicle size, thickening of vaginal wall and increased uterine vascularity. Estrus is a period of receptivity to the male and lasts for 18-24 hr with some variation among breeds. Ovulation occurs spontaneously about 10 hr after the end of estrus (Cupps et al., 1969). Metestrus is the post-ovulatory phase during which formation of corpus luteum takes place. Progesterone, a hormone secreted by the corpus luteum, prevents occurrence of further heat periods. Estrus does not occur so long as an active corpus luteum is present. Diestrus is the short period of quiescence between estrous cycles.

Most of the early work reported on the control of estrus utilized daily injections of progesterone (Christian and Casida,

1948; Willet, 1950; Ulberg et al., 1951; Hansel and Trimberger, 1952; Dziuk, 1966). The duration of the treatment lasted 13 to 18 days. About 48 to 72 hrs after cessation of progesterone treatment estrus began with ovulation occurring 36 to 48 hrs later (Dziuk, 1966). Nellor and Cole (1956), in an attempt to overcome the practical disadvantages of daily injections, reported that a single injection of 560 mg progesterone in starch emulsion, followed in 15 days by a single injection of 750-2140 IU of equine gonadotrophin (PMS), resulted in 90% of the normally cycling heifers showing estrus 1-4 days after PMS injections.

A wide variety of potent orally active compounds inhibits estrus in bovine (Baker and Coggins, 1968; Dhindsa et al., 1967; Fahning et al., 1966; Menge and Christian, 1968; O'Brien and Zimbleman, 1970; Wiltbank and Kasson, 1968; and others). Orally administered medoxy progesterone 17-acetate (MAP), when given daily at levels of 180-200 mg, was effective in inhibiting estrus and ovulation (Hansel and Malven, 1960; Dhindsa et al., 1967). Chlormadinone acetate (CAP) was equally effective when only 10-20 mg was administered daily (Wagner et al., 1968). A third compound, melengestrol acetate (MGA), was even more potent orally and the effective dose was as low as 0.5 mg daily (O'Brien and Zimbleman, 1970; Roussel and Beatty, 1969; Smith and Zimbleman, 1968). Six-methyl-17-acetoxy progesterone (Repromix) when

fed twice daily at a dosage level of 180-400 mg also successfully inhibited estrus (Zimbleman, 1963; Nellor , 1960). High cost of feeding hormones, variability in estrous response and the practical problem of feeding large number of animals in a herd resulted in oral administrations of hormones being impractical for the producer.

A great deal of effort has been expended to develop a subcutaneous implant or vaginal pessary that would allow for passage of constant and continuous amount of progestagen for the duration of the cycle. Dziuk et al. (1968) used silicone subcutaneous implants to control estrus in ewes. The implants were impregnated with melengestrol acetate (MGA) and kept in place under the skin of axilla for 14 to 45 days. Ninety-five per cent of the estrous cycles were inhibited and 75% of the ewes were in estrus between 36 and 54 hours after implant removal. The conception rates and number of lambs born from treated ewes did not differ from controls. The use of MGA implants in cattle have also been reported (Lauderdale et al., 1972). In a group of 32 heifers which had the implant in place subcutaneously for 14 days, 87.5% were in estrus two to six days after implant removal. Less precise synchronization was obtained if the implants were in place for 28 days. Estrus was also successfully synchronized using subcutaneous implants containing Norethandrolone (NILVAR) (Wiltbank et al., 1971) and

progesterone (Roche, 1974). In spite of the high success rate, the large surface area required to release sufficient progesterone to block estrus in cattle does not favour their widespread use.

Robinson (1964,1965) reported synchronization of estrus and ovulation in sheep following removal of intravaginal sponges impregnated with 17α-acetoxy-fluoro-11β hydroxy progesterone (cronolone). Further work (Robinson, 1967) has shown that this technique enables simple and precise control of ovarian function in sheep. The retention rate of intravaginal sponges in cows and heifers has been variable (Carrick and Shelton, 1967; Wishart and Hoskin, 1968; Scanlon et al., 1972; Sreenan, 1974) and is therefore impractical for estrous synchronization in cattle. However, silastic vaginal coils containing progesterone have been shown to suppress estrus and ovulation in cattle and the retention rate was reported to be excellent (Mauer et al., 1975; Roche, 1975).

In an attempt to achieve a more precise synchronization and ovulation following progesterone treatment, various other hormones were administered after termination of progestin treatment. Baker and Coggins (1968) injected 1000 IU of HCG into beef heifers 48 hours after the last 6 chloro-17 acetoxy progesterone (CAP) treatment and observed no significant effect on the incidence of estrus or conception compared to those with

CAP alone. Wiltbank et al. (1971) injected 2 mg of estradiol-17β into beef heifers 24 hours after removal of implants containing Norethandrolone (Nilvar) and found that 100% of the animals came into estrus within a 48-hr period and 100% ovulated within a 36-hr period after implant removal. Similar synchronization results were obtained by Hansel and Schechter (1972) using MAP and estradiol benzoate. Mauer et al. (1975) injected 100 µg of gonadotrophin releasing hormone (GnRH) 30 hrs following 21-day progesterone treatment and observed 90% ovulated within 69 hrs after withdrawal of progesterone treatment. They reported a conception rate equal to or better than the same number of controls following progesterone and GnRH treatment.

In spite of the successful synchronization observed, the fertility rate following synchronized estrus was reported to be variable and generally low (Hansel, 1967; Johle, 1972). The reduced fertility has been attributed to changes in quality and quantity of cervical mucus (Johnson and Ulberg, 1965; Hill et al., 1971), changes in body temperature (Johnson and Ulberg, 1965; Long et al., 1969), changes in time required for sperm capacitation (Lauderdale and Erickson, 1970), histological changes in endometrium (Wordinger et al., 1971), altered transport of ova through the oviduct (Reed and Rich, 1972, changes in the cytochemical characteristics of granulosa cells (Kruip and Brand, 1975), high progesterone concentration three or four days before

synchronized estrus (Henricks et al., 1970; Britt and Ulberg, 1972), and higher estrogen surge at synchronized estrus than that observed in untreated cows at the time of estrus (Hackett et al., 1972).

Fertility rate comparable to controls was obtained in heifers synchronized by feeding a progestagen for 9 days and injecting estradiol-17β on the second day of progestagen treatment (Wiltbank and Kasson, 1968). Estrogen was administered to cause early regression (Wiltbank et al., 1961; Kaltenbach et al., 1964) progesterone to block estrus and ovulation (Hansel et al., Lamond, 1964). Similarly, high fertility rate has also been reported by using short term (9-12) days) subcutaneous progestagen implant treatment and an injection of estrogen at insertion (Roche, 1974; Wiltbank and Gonzalez-Padilla, 1975).

The results with estrogen, progesterone sequential treatment are encouraging. One possibility of overcoming the practical problem of using subcutaneous progestagen implant and an injection of estradiol benzoate is to develop a short term vaginal device to release estradiol for the first two days of treatment and progesterone for a period of 9-12 days.

Another approach to estrous synchronization is to cause rapid luteolysis by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Administration of $PGF_{2\alpha}$ to cows between day 5 and 16 of the estrous cycle was followed by a decrease in serum progesterone (Louis et al., 1973;

Rajamahendran et al., 1976) and returned to estrus within 72 hrs (Rowson et al., 1972; Lauderdale, 1972; Louis et al., 1973; Inskeep, 1973; Rajamahendran et al., 1976). The fertility following induced estrus appears to be normal (Rowson et al., 1972; Inskeep, 1973; Lauderdale et al., 1974). A major problem in this treatment is that cows in first five days of the cycle do not respond to $PGF_{2\alpha}$. Double injection of prostaglandin 10-12 days apart without reference to previous natural estrus resulted in the majority of animals returning to estrus 48 to 72 hours after the second injection and the fertility based on small number of animals appeared to be normal (Cooper and Furr, 1974; Lauderdale, 1974; Rajamahendran and Baker, 1975).

Any consideration of the capabilities and limitations of estrogen plus progesterone method for cycle regulation needs to be prefaced by a consideration of current knowledge of blood hormone levels in normal animals. Several features of the data relate directly to estrous cycle control techniques. Plasma lutenizing hormone (LH) concentrations remain relatively low during the part of the cycle when plasma progesterone concentrations are high and relatively large peaks of short duration in plasma LH occur at about the time of estrus (Henricks et al., 1970; Snook et al., 1971; Echternkamp and Hansel, 1971). Three peaks in plasma estrogens are seen during the cycle in the cow (Henricks et al., 1971; Echternkamp and Hansel, 1971)

and each peak seems related to a period of accelerated follicular growth. One peak occurs early in the cycle and before plasma progesterone levels rise appreciably; another occurs after plasma progesterone declines and is probably a refleqtion of growth of the ovulatory follicle. The third peak appears just prior to corpus luteum regression. These data emphasize two things - (a) the central role of plasma progestefone levels in controlling the estrous cycle of normal animals, and (b) the possible role of estrogen in corpus luteum regression.

The role of estrogens and progesterone in corpus luteum function in the bovine has been examined by many workers. Greenstein et al. (1958) first reported that estradiol injected daily on days 2 to 12 inclusive of the estrous cycle caused early regression of the corpus luteum. Wiltbank et al. (1961) verified this observation and stated that single intramuscular injections of as little as 5 mg of estradiol valerate or 25 mg of estrone would cause early corpus luteum regression. Recently it has been reported (Lemon, 1975) that estrogens show a variety of effects on the bovine corpus luteum depending on the stage of the cycle at which they are administered. Luteal regression was observed only during luteal phase. Exogenous administration of progesterone early in the cycle before progesterone rises, also shortens the length of the estrous cycle and reduces corpus luteum weights in the cow (Harms and Malven,

1969; Ray et al., 1961; Woody et al., 1967). It was observed (Harms and Malven, 1969) that progesterone injections had to be started on day 1 and continued until day 3 for maximum effectiveness. If progesterone injections were not started until day 2, the injections had to be continued beyond day 3, in order to shorten the cycle length. Woody and Ginther (1968) also showed that there was greater reduction in estrous cycle length when progesterone injections were started on the day of estrus than when started two days later.

The synchronization of estrous behaviour with ovulation is necessary for fertilization and establishment of successful pregnancy. From extensive studies in the ovariectomized ewe (Robinson, 1959; Scaramuzzi, 1968;1971), it is now understood that both progesterone and 17β-estradiol play an important role in the induction of estrous behaviour. Progesterone priming in the ovariectomized ewe has been shown to reduce the median effective dose of estrogen necessary to induce estrous behaviour (Robinson, 1959), to decrease the time between injection and onset and to increase the duration of estrous behaviour (Scaramuzzi, 1971). However, if levels of progesterone remain elevated at the time of estrogen surge, estrous behaviour is completely inhibited (Robinson, 1959; Short et al., 1973). The progesterone block of estrous behaviour is most likely at the hypothalamic level, because estrous behaviour can be induced in

estrogen-treated castrated females regardless of the presence of pituitary gland (Astwood and Dempsey, 1941; Dey et al., 1942).

Since luteinizing hormone (LH) has been often suggested to be the pituitary hormone directly responsible for ovulation in the cow, considerable interest centered on the role of progesterone and estrogen in the release of LH. Several researchers (Howland et al., 1971; Hobson and Hansel, 1972; Short et al., 1973; Hausler and Malven, 1976) were able to reproduce the plasma preovulatory LH surge in the ovariectomized cow by administration of estradiol. Intramuscular injection of 400 μg of estradiol benzoate resulted in LH surge approximately 42.0 hr post-injection (Hobson and Hansel, 1971). An unphysiological dose of 10 mg 17β-estradiol resulted in an LH surge 20-26 hr post-injection (Howland et al., 1971; Short et al., 1973; Hausler and Malven, 1976), which may indicate that latent period to be dose dependent. A large number of similar experiments conducted in monkey (Karsch et al., 1971), rat (Ferin et al., 1969) and sheep (Gording et al., 1969; Scaramuzzi et al., 1971) have given similar results.

Additional support for an estrogen stimulated LH release can be inferred from plasma estrogen concentrations (Echternkamp and Hansel, 1971; Henricks et al., 1971; Christensen et al., 1971) and urinary estrogen excretion values in the cow (Garverick et al., 1971). Echternkamp and Hansel (1971)

observed a rise in estradiol and estrone values prior to estrus with the maximum mean value at estrus. A LH peak occurred on the day of estrus. Henricks et al. (1971) measured total estrogen in samples collected twice daily four days prior to estrus, the levels are in agreement with the previous investigations. Christensen et al. (1971) also measured total estrogen in bovine blood at four-hour intervals and found estrogen levels highest approximately 24 hours before the LH peak. Garverick et al. (1971) reported an increase in the urinary excretion of estradiol-17α in the cow two to three days prior to estrus with the peak value occurring on the day of estrus in most instances.

The mechanism of action of estrogen in gonadotrophin release is not well understood. Estrogen increases LH release in the ewe (Radford et al., 1969; Caldwell et al., 1970). The latter workers provided additional evidence that estradiol was essential for inducing the LH surge, since animals immunized against estradiol-17β-hemisuccinyl-BSA did not show any peak of LH following the injection of 17β-estradiol. Evidence for hypothalamic control of luteinizing hormone via the action of luteinizing hormone releasing factor on the anterior pituitary has been reported in the rat (Schneider and McCann, 1970), sheep (Reeves et al., 1971) as well as cow (Hackett and Hafs, 1969). However, the sites of action of estrogen on LH release are not well defined, but it appears to have an action on both

hypothalamus and anterior pituitary level as indicated by the presence of specific binding sites in both pars distalis and anterior hypothalamus (Kato and Villee, 1967) of the rat. There is also evidence that estrogen receptors exist both in the hypothalamus and anterior pituitary. Thus tritiated hexostrol was shown to be concentrated in the anterior pituitary of goat and sheep (Galscock and Hockstra, 1969). Although uptake of estrogen by the hypothalamus is quite low relative to that by the pituitary, investigation by both biochemical (Kato and Villee, 1967; Parker and Mahesh, 1976) and autoradiographic methods (Stumpf, 1968) demonstrated receptor sites for estradiol in the hypothalamus.

The role of progesterone in the control of estrus and ovulation is not well defined in the bovine. Earlier studies indicate that progesterone may be involved in the induction of estrous behaviour, LH release and ovulation in the cow. The above view is based on the presence of progesterone in bovine follicular fluid near the time of ovulation (Edgar, 1963), while the injection of 5-15 mg of progesterone at the beginning of estrus significantly hastened the time of ovulation. Later studies (Stabenfeldt et al., 1969; Henricks et al., 1970; Kazama and Hansel, 1970; Katangolle, 1974) have failed to establish a preovulatory progesterone rise in the cow. In contrast Ayalon and Shemesh (1974) recently reported a preovulatory rise of

plasma progesterone in bovine.

Exogenous administration of progesterone to cycling cows (Hobson and Hansel, 1971) and ovariectomized cows (Short et al., 1973; Hausler and Malven, 1976) did not have any influence on blood LH levels. Hansel and Malven (1970) suggested that the decline in progesterone to less than 1 ng/ml of plasma is essential for the occurrence of preovulatory rise in LH in cycling cows. This is based on the observation in cows with an extended cycle (25 days) where the preovulatory LH rise did not occur until after progesterone values declined below this level. The mechanism by which progesterone inhibits estrus, LH release and ovulation in the bovine is not clear. It has been observed that progesterone will inhibit estrogen mediated LH release in intact ewe (Bolt et al., 1971), ovariectomized ewes (Scaramuzzi et al., 1971; Diekman and Malven, 1973) and ovariectomized monkeys (Yamaji et al., 1970), which suggests that progesterone inhibits the effect of estrogen on inducing LH release. In support of this mechanism in bovine Hobson and Hansel (1970) observed that estrogen-induced LH release was inhibited in luteal phase heifers. However, others (Short et al., 1973; Hausler and Malven, 1976) failed to inhibit estrogen-induced LH release with progesterone in ovariectomized heifers. In view of the conflicting results, further work is necessary to define the mechanism of progesterone action in the control of

estrus and ovulation in the bovine. One way of testing would be to attain a continuous physiological plasma estradiol and progesterone level in ovariectomized heifers and to observe whether progesterone is able to overcome the LH release induced by estradiol.

Puberty in farm animals has been defined in a number of ways. Marshall (1922) used the criterion of occurrence of "those constitutional changes whereby the two sexes become fully differential". Crew (1931) pointed out puberty and sexual maturity were not the same. In agreeing with this view, Asdell (1946) defined puberty as the time at which reproduction first becomes possible and sexual maturity as the time the animal reaches its full reproductive capacity. Studying the effect of constant environmental temperature on appearance of puberty in beef heifers, Dale et al. (1959) measured puberty by the appearance of persistant structures - follicles and CL on the surface of the ovary by rectal palpation. Others (Reynolds et al., 1963; Wiltbank et al., 1959; Kaltenbach and Wiltbank, 1962; Bellows et al., 1965) determined age and weight at puberty in heifers with respect to the first ovulatory estrus, while Hawk et al. (1954) considered puberty in Holstein heifers on the basis of first estrus. Manifestation of estrus alone does not indicate that reproduction is possible; ovulation and development of functional corpus luteum must follow such an

estrus at which the animal exhibits her first ovulatory estrus. Therefore, puberty in heifers should be viewed as the age and weight at which the animal exhibits her first ovulatory estrus.

In the prepuberal heifer follicles are formed before puberty, but they do not mature and rupture until puberty is reached (Hammond, 1927). Attainment of puberty in heifers is affected by breed (Jobert, 1963; Reynolds, 1963; Plasse et al., 1968), level of nutrition (Jobert, 1963; Wiltbank et al., 1969) and environment (Dale et al., 1958;1959; Plasse et al., 1968). Unlike most mammals heifer calves respond to the administration of ovarian and gonadotrophic hormones virtually from birth (Casida et al., 1943; Rowson, 1951; Marden, 1953 and others). This indicates that puberty might be influenced by hormone treatment. The understanding of the hormonal status of the prepuberal bovine is necessary to achieve this objective.

Desjardins and Hafs (1968) have studied the pituitary levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) of heifers from birth to puberty. Their results show that puberty is associated with decreased levels of pituitary LH followed by an almost continuous increased level towards the next estrus. Pituitary FSH content decreases relatively less than and somewhat before LH during the week before estrus.

Changes in the plasma progesterone concentration were studied in heifers at puberty (Donaldson et al., 1970). Plasma progesterone concentration of 12 cycling heifers in which estrus was not previously been detected, ranged from 0.3 to 3.9 ng/ml when the first blood was obtained. Seven of these heifers did not have a CL at palpation and five of the seven heifers had high plasma progesterone concentrations. They suggested that at puberty, first estrus was preceded by partial luteinization of the follicle rather than by ovulation without estrus and formation of CL.

Serum LH and prolactin were measured in heifers bled monthly from seven months of age until first estrus (Swanson et al., 1972). LH concentration increased as the first estrus approached, then declined, being lowest during mid-cycle. Prolactin levels before first estrus were higher than during subsequent cycles.

The interrelationship in levels of serum LH, FSH, GnRH, prolactin, estradiol-17β and progesterone occurring in the blood of heifers before and at the onset of puberty was recently reported by Gonzalez-Padilla et al. (1975). They made the following observations - (a) There were no marked changes in FSH, prolactin or GnRH concentrations as puberty approached or during the first cycle. (b) During the prepuberal period LH concentration fluctuated markedly with an average concentration higher than

that observed during the first cycle. In addition to pubertal peak of LH (day 0), each heifer showed another peak of LH of similar duration and magnitude between days -11 to -9.(c) Concentrations of estradiol-17β were high (about 20 pg/ml) prior to day 40, then decreased gradually for three to four days to a level that was rather constant through the remainder of the sampling period. There was no elevation of estradiol-17β associated with LH peak. (d) Progesterone concentrations were very low in the prepuberal period (0.3 ng/ml), but there were two distinct elevations in every heifer before day 0. The return of the first elevation to baseline concentration was always followed by the first peak of LH, while the second peak preceded the pubertal peak of LH.

To summarize, the hormonal changes occurring at puberty in heifers have been presented (Desjardins and Hafs, 1968; Donaldson, 1970; Swanson et al., 1972; Gonzalez-Padilla et al., 1975). Results suggest there are decreased levels of pituitary LH and a corresponding increase in serum LH concentration as the first estrus approached. Pituitary FSH concentration decreases at puberty but no marked change occurred in the blood. GnRH concentration in blood did not differ at puberty. Levels of estradiol were high 40 days prior to onset of puberty then gradually decreased to a level that was rather constant until puberty was reached. No elevation in estradiol

concentration was associated with pubertal peak of LH. There were two distinct elevations in progesterone concentration before puberty. The return of the concentration was always followed by a peak of LH. These observations indicate that progesterone is involved in the final events leading to puberty.

The subject of induction of puberty in heifers has received great attention during the past fifteen years. Howe et al. (1962) first showed that estrus and ovulation can be induced by progesterone-PMSG-HCG-treatment. Using various combinations of progesterone, estradiol-17β, PMSG and HCG by injections to induce estrus and ovulation in six-month old prepuberal calves, the same authors observed inconsistent results.

and HCG caused hyperfollicular development. However, pretreatment with progesterone reduced the number of small follicles and increased the number of medium and large size follicles and in some cases caused ovulation. Estradiol-17β, following PMSG suppressed follicular growth. Similarly Neville and Williams (1973), using various combinations of estradiol and PMSG in yearling heifers primed with progestagens for either 9 or 18 days, observed inconsistent results. Estrus occurred in 50 to 100% of the treated heifers and conception rate was very low.

Arije et al. (1969) reported a treatment regime in which Hereford heifers, aged 256 to 296 days, were pretreated with a subcutaneous implant containing 200 mg progestagen for 15 days

before PMSG administration. In addition, estradiol-17β was injected to some heifers at the time of insertion of implant, some were injected at the time of PMSG injection and the rest received both at insertion and PMSG administration. The percentage of heifers showing estrus and ovulation were 78, 60 and 50 for progesten-PMSG, estradiol-progesten-PMSG and estradiol-progesten-PMSG-estradiol, respectively.

Induction of puberty in 14-15 month old Angus heifers with progesterone-estradiol combination was recently reported (Gonzalez-Padilla et al., 1975). They observed that progesterone treatment alone did not induce estrus nor caused a significant alteration in serum LH and FSH concentration. Injection of estradiol-17β alone resulted in estrus behaviour and an LH peak, but none ovulated. In heifers receiving estradiol-17β 48 hours after progesterone injection, one out of four heifers showed heat, all had LH peaks and three out of four heifers developed C.L. These authors also reported that fertile estrus in 12-14 month old prepuberal heifers could be achieved by treating them with either (a) single intramuscular injection of estradiol valerate plus 3 mg of progestagen (Norgestomet) at time zero, and a nine-day treatment of ear implant containing Norgestomet; or (b) daily injection of progesterone for four days plus 5 mg of estradiol valerate on day 1 of progesterone injection and 2 mg of estradiol valerate two days after

termination of progesterone injection. More than 90% of the heifers treated exhibited estrus four days after implant removal. They also obtained a fertility rate of about 60% with treatment "a" in an extensive field trial.

Using various treatment regimens with GnRH and with or without PMSG, it was observed (Mellin et al., 1975) that GnRH alone generally stimulated follicular growth in calves but in most instances did not result in ovulation. However, GnRH did induce ovulation in all PMSG primed calves.

A review of existing literature indicates that cyclic activity can be induced in prepuberal heifers by proper combination of hormonal treatments. From all reports it also can be seen that increased length of progestagen administration (priming) had an advantage in the induction of estrus and ovulation. The recent report (Gonzalez-Padilla et al., 1975) on the initiation of fertile estrus in prepuberal heifers using estrogen-progestagen combination treatment is very encouraging. However, the heifers used in their study were about 12 to 14 months of age and it may be that their treatment may not be effective in heifers far from reaching puberty. Therefore, further research is indicated to develop a satisfactory treatment for the initiation of cyclic activity in prepuberal heifers.

The hormonal events associated with natural onset of puberty are well documented in rats compared to other

species. In rats, administration of estrogens (Ramirez and Sawyer, 1965; Smith and Davidson, 1968) and aromatizable androgens (Knudsen and Mahesh, 1975) advanced the onset of puberty. Presl et al. (1969) reported an increased serum level of estradiol near the time of puberty on the female rat. Evidence for secretion of estrogens on day 32 of life in female rats was obtained on the basis of increased uterine weight, changes in endometrial luminal epithelium, etc. (Knudsen et al., 1974). He suggested that this secretion of estrogens presumably triggered first ovulation on day 38 and occurred prior to measurable increases in serum FSH and LH. The mechanism by which steroids may modulate the secretion of gonadotrophin is not known. It has been demonstrated by the work of several investigators that both the anterior pituitary and hypothalamus possess cytosol receptors for estradiol. A decrease in cytoplasmic receptors for estradiol in the hypothalamus, pituitary and uterus of the rat has been demonstrated after a single injection of estradiol (Cidlowski and Muldoon, 1974; Sarff and Gorski, 1971; Mester and Bauliue, 1975). An enhancement of pituitary responsiveness to exogenous LH-RH on proestrus in the rat has been demonstrated (Aiyer and Fink, 1974). This increased responsiveness has been correlated with depletion of cytoplasmic estrogen receptors of the pituitary and hypothalamus (Greeley et al., 1974;1975). Evidence presented here suggests that estrogens serve as the

primary trigger for setting up the sequence of events leading to puberty in the rat.

Studies on the induction of puberty and the blood hormone concentration profile at the onset of natural puberty in prepuberal calves suggest that progesterone may be involved in the final events leading to puberty. In determining the value of progesterone as an aid in inducing estrus and ovulation in anestrous ewes, Robinson (1959) indicated that progesterone conditioned receptors in higher centres to respond to low levels of estrogen. One possible way by which progesterone may influence the final event leading to puberty in prepuberal heifers is as follows. Short term progesterone priming may increase the cytoplasmic estrogen receptors at the anterior pituitary and/or hypothalamus. This in turn will increase the sensitivity of the receptors for blood estradiol, resulting in depletion of cytoplasmic estradiol receptors, which in turn initiates gonadotrophin surge and ovulation.

The present study had four main objectives. The first was to develop a relatively simple device to administer progesterone continuously in prepuberal heifers and to induce estrus, ovulation and cyclic activity in these progesterone primed heifers with 17β-estradiol, PMSG or GnRH. The second objective was to develop a progesterone and progesterone + estradiol-17β silastic vaginal device to induce estrus, ovulation

and fertility in cycling heifers. The third objective of this study was to determine the interaction between estrogen and progesterone in eliciting estrus response and LH release in ovariectomized heifers. Fourthly the effect of short-term progesterone and estradiol plus progesterone on the presence of cytoplasmic progesterone and estrogen receptors in hypothalmus, anterior pituitary and reproductive organs were studied in prepuberal heifers.

EXPERIMENTS AND RESULTS

EXPERIMENT I. EFFECT OF PROGESTERONE PRIMING ON INDUCTION OF ESTRUS, OVULATION AND CYCLIC ACTIVITY IN PREPUBERAL HEIFERS

Introduction

A review of existing literature indicates that cyclic activity can be induced in prepuberal heifers by proper combination of hormonal treatment. However, a satisfactory and reliable method is not yet available to induce cyclic activity. From all reports it also can be seen that an increase in the length of progestagen administration (priming) had an advantage in the induction of estrus, ovulation and cyclic activity in prepuberal heifers. Daily injections and oral administration of progestagen are not practical. In 1966, Dziuk and Cook reported that steroids pass through the walls of silicone rubber tubing. Steroids administered continuously from silastic implants provide biological effects at a lower dose rate than oral administration (Kincl and Rudel, 1971).

The objectives of this experiment were to study the effect of surface area and route of administration of silastic devices containing progesterone on concentrations of serum progesterone, and to induce estrus, ovulation and cyclic activity following progesterone priming with estradiol-17β, pregnant

mare serum gonadotrophin or gonadotrophin releasing hormone in prepuberal heifers.

Materials and Methods

Thirty-two prepuberal heifers, six to eight months of age and weighing approximately 250 kg, were used. The experiment was conducted during two seasons. The first group of 16 heifers was treated during winter (January) in tie stalls with artificial 12 hr light and 12 hr darkness. They were fed a maintenance ration of grain and hay. The second group was treated during summer (July). These heifers had free access to pasture and were supplemented with grain. All heifers were palpated rectally prior to the commencement of treatment and none were cycling.

Four heifers in each group were randomly allocated to one of the following four treatments.

Treat-A- Single silastic tubing (surface area=60 cm^2) containing 1 g of progesterone inserted subcutaneously.

Treat-B- Double silastic tubing (surface area=120 cm^2) containing 2 g of progesterone inserted subcutaneously.

Treat-C- Double silastic tubing (surface area=120 cm^2) containing 2 g progesterone and tied together as shown in Figure 1, and inserted intravaginally.

Treat-D- Silastic rubber impregnated with 6.6% progesterone

Figure 1. Illustration of devices used in Experiment I.

A. Single silastic tubing containing 1 gram of progesterone (surface area = 60 cm^2).

B. Double silastic tubing containing 2 gram of progesterone (surface area = 120 cm^2).

C. Double silastic tubing containing 2 gram of progesterone and tied together (surface area = 120 cm^2).

D. Abbott's coil - silastic rubber impregnated with 6.6% progesterone (surface area = 210 cm^2).

(surface area=210 cm^2) and inserted intravaginally.

Preparation and Insertion of Devices

Treatment "A", "B" and "C" devices were made using silicone rubber tubing with an inside diameter of 0.79 cm and an outside diameter of 1.27 cm (Dow Corning, Silastic No. 601-501). The tubing was cut into 15 cm lengths, sealed at one end with medical adhesive (silastic brand, Dow Corning) and allowed to harden for 12 hrs. One gram of progesterone (Sigma) dissolved in ether was then poured into each tube. The ether was allowed to evaporate and the open end was sealed with medical adhesive. Abbott Laboratories kindly supplied the devices for treatment "D". The device consisted of a coil of stainless steel covered with silastic rubber impregnated with 6.6% progesterone. The devices used in each treatment were weighed prior to insertion.

Subcutaneous devices were placed caudal to the shoulder joint, one on each side in treatment "B". The area was clipped, scrubbed and a disinfectant was applied before making an incision in the skin. With the aid of a trocar the device was inserted leaving a string which was attached to the device protruding from the incision. The incision was closed with silk sutures. Devices were removed by making a small incision and pulling the string attached to the device. Intravaginal devices were inserted into the vagina with a plastic speculum and

plunger. The external genital area was scrubbed and a disinfectant was applied before inserting the device. The devices were removed by pulling the string attached to the device. The devices used in each treatment were left in place for a period of 20 days. Following removal, devices were cleaned, dried and weighed.

Blood Samples

Ten ml of blood was collected from the jugular vein at 0600 hr, 1200 hr, 1800 hr and 2400 hr on day -1, 1, 4, 8, 12, 16 and 20 (day 0 = day of insertion of device). Blood samples were also collected at 1200 hr on day 1, 2 and at 6, 12 and 24 hrs after removal of the device. The blood samples were centrifuged and the serum was stored at -20°C in small aliquots until assay. Haematocrit values from 0600 hr, 1200 hr, 1800 hr and 2400 hr samples collected on day -1, 4, 8, 12, 16 and 20 in sixteen heifers, treated in January, were determined by microcapillary tube method.

Hormonal Treatment Post Device Removal

Immediately after removal of the device the heifers were palpated and one heifer in each of the four treatment groups received saline, 5 mg estradiol-17β (Sigma), 500 IU of pregnant mare serum gonadotrophin (PMSG, Ayerst Laboratories) or 100 μg

of gonadotrophin releasing hormone (GnRH, Abbott Laboratories). The heifers were observed frequently for estrus, with an aid of a vasectomized bull and Kamar heat mount detectors. All the heifers were palpated for the presence of a corpus luteum (CL) eight days after device removal. Heifers with palpable corpus luteum were also bled on day 8, 13, 18 and 23 days after removal of the device. These heifers were observed for signs of estrus during the sampling period and rectal palpations were also done at day 23 after removal of device to assess ovarian activity.

Radioimmunoassay for Progesterone

The radioimmunoassay as described by Rajamahendran et al. (1976) was used to quantitate serum progesterone in heifers with a slight modification. After extracting with petroleum ether, the serum portion was frozen at -20°C for one hour and the solvent (extract) was decanted into assay tubes. The progesterone antibody was kindly supplied by Dr. K. Roberts of the University of Montreal.

Statistical Analyses

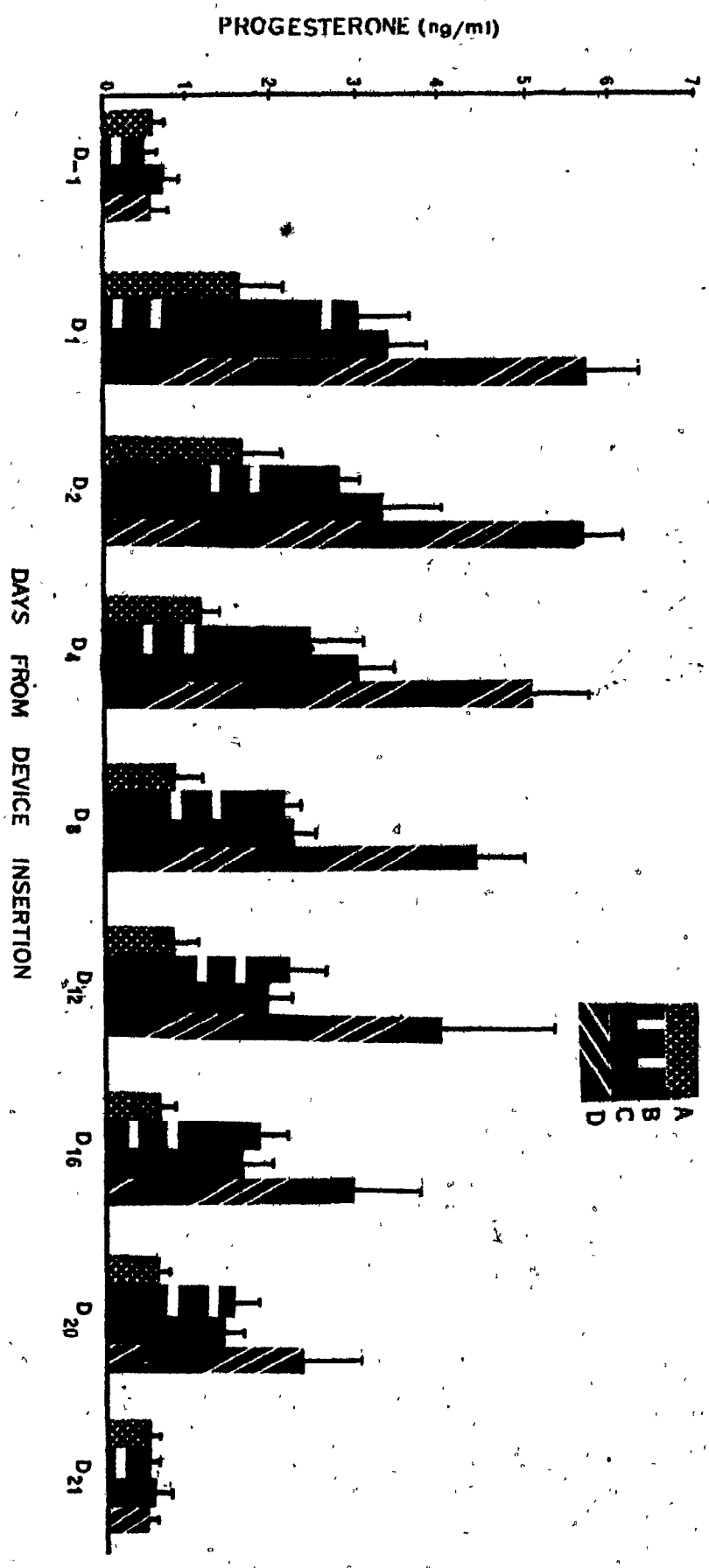
Progesterone data were analyzed statistically as a split split plot experiment in a completely randomized design. Seasons were considered as mainplot units, day time combinations were considered as subplot units and treatments as sub-subplot units. The means were analyzed using LSD. (Steel and Torrie, 1960).

Results

The mean concentration of serum progesterone before, during and after the 20-day progesterone treatment period is shown in Figure 2. The pretreatment values were not different ($P > 0.05$) among treatment groups (0.6 ng/ml). In treatment "A" heifers, the serum progesterone rose to a maximum value of 1.6 ng/ml one day after insertion of the device. This value was higher ($P < 0.01$) than pretreatment concentration and lower ($P < 0.05$) than for heifers in treatments "B", "C" and "D". Thereafter the serum progesterone remained constant on day 2 and gradually declined during the sampling period and reached pretreatment values eight days after inserting the device.

Mean serum progesterone in treatment "B" heifers was elevated to 3.0 ng/ml on day 1. This value was higher ($P < 0.01$) than pretreatment concentration and that observed in treatment "A" heifers, lower ($P < 0.05$) than for heifers in treatment "D", but did not differ from treatment "C" heifers. Serum progesterone in treatment "B" heifers thereafter decreased gradually during the sampling period and reached a value of 1.5 ng/ml on the day devices were removed. Progesterone decreased significantly ($P < 0.01$) 24 hours after removal of the device. The mean concentration of serum progesterone following treatment "C" followed a similar pattern as in treatment "B". However, the

Figure 2. Mean serum progesterone before, during and after insertion of a single subcutaneous ("A"), double subcutaneous ("B"), vaginal ("C") or Abbott's ("D") progesterone devices in prepuberal heifers. n = eight heifers per treatment. D_{-1} = day before insertion of the devices. Devices were removed on D_{20}. Analysis of variance of serum progesterone showed that progesterone concentration varied with treatment ($P < 0.01$) and day of the treatment ($P < 0.01$).


PROGESTERONE (ng/ml)
0
1
2
3
4
5
6
7
D-1
D1
D2
D4
D8
D12
D16
D20
D21
DAYS FROM DEVICE INSERTION
A
B
C
D

concentration of serum progesterone in treatment "C" was slightly higher on day 1 (3.3 ng/ml) and day 8 (2.2 ng/ml) and slightly lower during the remainder of the sampling period, than for treatment "B" heifers. In treatment "C" serum progesterone also declined ($P < 0.01$) 24 hours after removal of the device.

Following treatment "D" the mean concentration of serum progesterone was elevated to a maximum value of 5.7 ng/ml on day 1. This value was higher ($P < 0.01$) than pretreatment levels and the comparable values (day 1 sample) for treatments "A", "B" and "C" treated heifers. After this maximal increase in progesterone, the concentration of serum progesterone declined gradually and reached 2.3 ng/ml at the time the device was removed. This concentration of 2.3 ng/ml was significantly ($P < 0.01$) higher than the corresponding values for treatments "A", "B" and "C". In treatment "D", as in treatment "B" and "C", a significant ($P < 0.01$) decrease in serum progesterone was observed 24 hours after removal of the device.

Analysis of variance of serum progesterone, from blood samples obtained on day -1, 4, 8, 12, 16 and 20, showed that progesterone concentration varied with treatment ($P < 0.01$), with the day of the treatment ($P < 0.01$) and time of the day ($P < 0.01$). Throughout the treatment period highest progesterone concentration was observed in treatment "D" heifers and lowest in treatment "A" heifers. No difference was observed between

treatment "B" and "C" heifers. The highest progesterone concentration (2.1 ng/ml) was observed at 0600 hr and the lowest (1.7 ng/ml) at 1800 hr. However, no difference ($P > 0.05$) was observed in the progesterone concentration between 1200 and 2400 hr (Appendix Table 3B). Analysis of variance also showed day by treatment ($P < 0.01$), day by time ($P < 0.05$) and treatment time ($P < 0.01$) interaction. Analysis of variance of hematocrit values for heifers treated in January revealed that hematocrit values were not affected by day or time of the day but they were affected by treatment (Appendix Table 3C).

All heifers were palpated on day 10 of treatment and at removal of the device to evaluate the uterine and ovarian activity. The uterus and ovaries were larger at this time (day 10) than pretreatment. Heifers in treatment "B", "C" and "D" had an enlarged turgid uterus and enlarged ovaries compared to treatment "A" heifers during treatment and after removal of the device. Subcutaneous and vaginal devices were all retained by the heifers. A whitish yellow discharge was observed during the treatment period from all heifers in treatment "C" and "D". The devices from these heifers were coated with whitish yellow mucus at removal.

None of the heifers treated with saline, PMSG or GnRH following treatment "A" exhibited estrus or ovulated. However, both heifers treated with estradiol-17β were hyperactive and

showed standing estrus 48 hours post-injection, but neither ovulated. Hence data on "B", "C" and "D" heifers were combined to give six heifers per stimulation group (Table 1).

Table 1. Mean estrus response, ovulation, and serum progesterone concentration of progesterone primed prepuberal heifers to saline, estradiol-17β (5 mg), PMSG (500 IU) or GnRH (100 μg).

Parameters	Treatment			
	Saline	Estradiol-17β	PMSG	GnRH
Total no. of heifers	6	6	6	6
No. exhibited estrus	0	3	6	2
No. ovulated	0	2	6	2
Mean No. of ovulations	0	1	2.5	1
Mean serum progesterone (ng/ml) after removal of device				
D_8	-	1.8	2.8	1.9
D_{13}	-	3.7	5.6	3.8
D_{18}	-	3.9	5.4	3.9
D_{23}	-	2.0	4.6	2.0
No. returned to estrus 20-22 days after induced estrus	-	1	1	1

Of the saline, estradiol-17β, PMSG and GnRH treated heifers 0, 3, 6 and 2 showed estrus and 0, 2, 6 and 2 ovulated, respectively. One heifer in each of estradiol-17β, PMSG and GnRH treated group returned to estrus 20-23 days after induced estrus. Estradiol-17β treated heifers exhibited estrus approximately 24 hours earlier than PMSG or GnRH treated heifers. The range and

mean of ovulations in heifers receiving PMSG was 2 to 4 and 2.5 ovulations. In all groups the mean concentration of serum progesterone tended to increase during sampling period (D_8, D_{13}, D_{18}) and a decline in concentration was observed on D_{23}. The mean serum progesterone following PMSG treatment on D_8, D_{13}, D_{18} and D_{23} were higher ($P < 0.01$) than that of estradiol-17β or GnRH treated heifers. One heifer in each group had serum progesterone less than 1 ng/ml on day 23. Except for these three heifers others had palpable CL on this date.

EXPERIMENT II. EFFECT OF SILASTIC VAGINAL DEVICE CONTAINING PROGESTERONE AND PROGESTERONE + ESTRADIOL-17β ON ESTRUS CONTROL, OVULATION AND FERTILITY IN CYCLING HEIFERS

Introduction

The widespread application of control of estrus in cattle using progesterone requires a practical method of administering the steroid. Exogenous administration of progesterone to cows for the duration of a cycle controls estrus and ovulation, with most animals showing estrus two to five days post-progesterone withdrawal (Lamond, 1964). This method has been used widely but the conception rate at the resultant estrus has been low (Hansel, 1967). Fertility rate comparable to controls was obtained when heifers were synchronized by feeding a progestagen for nine days and injecting estradiol valerate on the

second day of progestagen treatment (Wiltbank and Kasson, 1968). Progestagens have been administered in the feed, in the drinking water, as injections or in implants. Practical problems of feeding, injecting or implanting progestagens can be overcome by intravaginal devices containing progesterone. Intravaginal pessaries have been tried in cattle but a variable retention rate has been reported (Carrick and Shelton, 1967; Scanlon et al., 1972; Sreenan, 1974).

In the previous experiment vaginal silastic devices containing progesterone were used to maintain serum progesterone greater than 1 ng/ml until removal. The serum progesterone profile during the estrous cycle in the bovine (Henricks et al., 1971; Wettemann et al., 1971; Rajamahendran et al., 1976) indicates that estrus and ovulation occurs only after the serum progesterone declines to less than 1 ng/ml. The objectives of this experiment were to determine the effect of (a) silastic vaginal device containing progesterone for 12 days plus an injection of estradiol benzoate and progesterone at insertion on estrus, ovulation and serum progesterone; (b) the stage of the estrous cycle at the time of insertion of vaginal device containing progesterone + estradiol-17β on estrus response and ovulation; and (c) silastic vaginal device containing progesterone + estradiol-17β on estrus response, ovulation, fertility, serum progesterone and estradiol, in cycling heifers.

Materials and Methods

The 59 Holstein heifers used were 16 to 18 months of age and weighed 280-380 kg. The heifers were maintained in a feed lot and were fed corn silage. All heifers except two had estrous cycles of 20 to 22 days and rectal palpation revealed the presence of normal ovaries and genital tracts. Mounting behaviour, Kamar heat mount detectors and rectal palpation of the tracts and ovaries were used to assess the onset of standing estrus and ovulation.

Group I

At zero time, 24 cycling heifers received an intramuscular injection of 200 mg of progesterone and 5 mg of estradiol benzoate in corn oil and an intravaginal device containing progesterone. The vaginal device remained in the vagina for 12 days. The estrus response was determined during and after removal of the device. Reproductive tracts were examined at removal and eight days later. Serum progesterone concentration was determined from 10 ml blood samples obtained from six heifers at zero time and 12 hours later. Other blood samples were taken on days 1, 2, 4, 8, 12 and 24 hours after removal of the device.

Group II

Vaginal devices containing progesterone + estradiol-17β

were inserted and maintained for 12 days at known stages of the estrous cycle. Eight heifers received devices between days 0 to 4, 5 to 15, and 16 to 20 of the estrous cycle. Heifers were observed for estrus during and after removal of the device. All heifers were palpated at eight days after removal.

Group III

At time 0, a progesterone + estradiol-17β vaginal device was inserted into the vagina of 11 cycling heifers. Two of these heifers (Nos. 277 and 278) had cystic ovaries and were given 100 μg of GnRH 48 hours before inserting the vaginal device. Twelve days later the devices were removed. The heifers were observed for signs of estrus during and after removal of the device. All heifers were inseminated with frozen semen whether or not they exhibited estrus 56 hours after removal of the device. In order to determine the serum estradiol-17β and progesterone profile, 20 ml of blood from the jugular vein were obtained from six heifers at -24 hr, 0 hr, 12 hr, days 1, 2, 4, 8, 12 and 24 hours after removal of the device.

Preparation and Insertion of Devices

Vaginal devices were made using silicone rubber tubing with an internal diameter of 0.79 cm and an external diameter of 1.27 cm (Dow Corning, Silastic No. 601-501). The tubing was

cut into 20 cm lengths, sealed at one end with silicone medical adhesive and allowed to harden for 12 hours. One gram of progesterone dissolved in ether was poured into each piece of tubing. The ether was evaporated and the other end was sealed with silicone medical adhesive. The two pieces of tubing were tied together to make the vaginal device (Figure 3). The total surface area of each vaginal device was 160 cm^2. Progesterone + estradiol-17β devices were prepared in the following manner. Ten mg of estradiol-17β were mixed with approximately 1 ml of silicone medical adhesive. The estradiol-17β mixture was used to coat about 40 cm^2 of the four ends of previously described progesterone vaginal device. The device was inserted into the vagina with a plastic speculum and plunger (Figure 3). The device was removed by pulling on a string attached to the device and left protruding from the vulva.

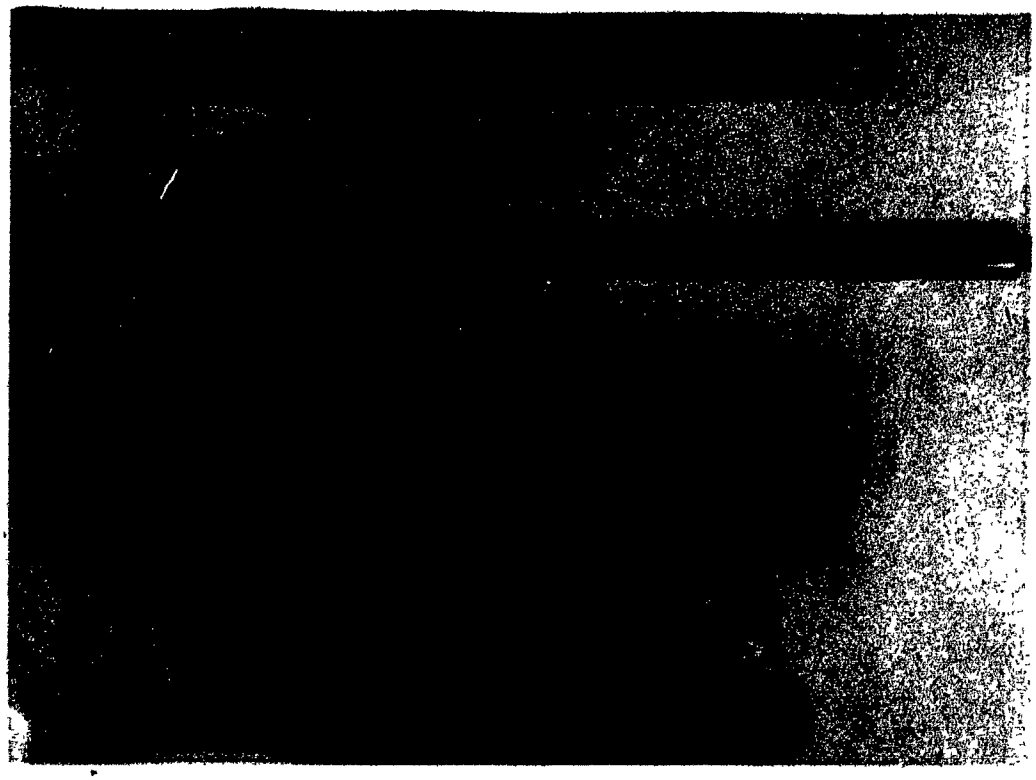
Radioimmunoassay for Progesterone and Estradiol-17β

Serum progesterone was measured by the procedure described in Part I. The radioimmunoassay as described by Korenman et al. (1974) was used with some modifications to quantitate serum estradiol-17β in heifers. All solvents used were reagent grade and purified before use. The estradiol-17β antibody was kindly supplied by Dr. G.D. Nisewender of Colorado State University, Fort Collins, Colorado. It was used at a dilution of 1:8,000

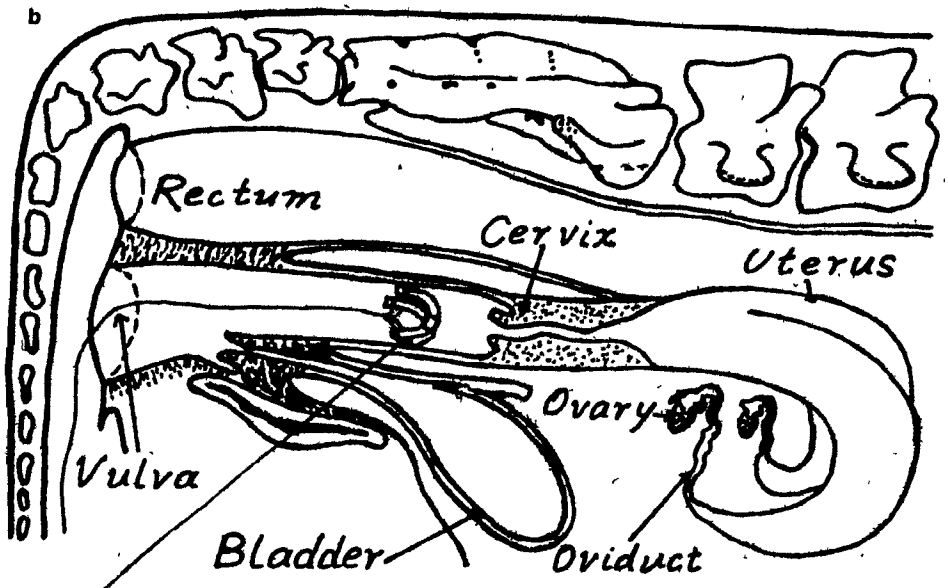
Figure 3a. Silastic vaginal device containing progesterone + estradiol-17β and equipment used for insertion of the device.

Figure 3b. Diagramatic representation of the bovine reproductive tract with silastic vaginal device in position.

a



b


Rectum
Cervix
Uterus
Ovary
Vulva
Bladder
Oviduct
Silastic Vaginal Device

in 0.1% gelatin buffer (0.1 M phosphate, pH 7.4). The same diluent was used for preparing estradiol-17β-2,4,6,7-^{3}H (20,000 DPM/0.05 ml). Labelled steroids were obtained from New England Nuclear Corporation. Estradiol-17β standards were prepared in benzene:methanol, 9:1. All the above solutions were stored at 4°C.

The detailed procedure consists of the following steps. The serum sample (1 ml) was extracted with 6 ml of freshly opened anhydrous diethyl ether in a 15 x 125 mm Teflon-lined screw cap extraction tube (Kimax brand). The mixture was shaken on a horizontal Eberback shaker for 10 minutes and then centrifuged (1,250 g) for two minutes. After extracting, the serum portion was frozen at -20°C overnight and the solvent (extract) was decanted into assay tubes (12 x 75 mm). The tube was then evaporated to dryness under a gentle stream of nitrogen in a sand bath at 40°C. The tube was rinsed down with 0.1 ml of methanol. The following standards were prepared in duplicate, 0,5,10, 20, 40, 80, 160 and 320 pg in 0.1 ml benzene:methanol. The extracted samples and the standards were evaporated to dryness. To the tubes was added 0.2 ml of phosphate buffer, 0.05 ml of ^{3}H estradiol-17β (20,000 DPM) and 0.05 ml of antiestradiol-17β (1:8000). The tubes were vortexed for five seconds and allowed to stand at 4°C overnight. Following this incubation period, 0.5 ml of freshly prepared dextran coated charcoal was added to

separate the bound and unbound fractions. The tubes were vortexed for five seconds and allowed to stand at 4°C for 15 minutes, centrifuged at 1,250 g for 20 minutes at 4°C. The supernatant was decanted into scintillation vials to which 10 ml of toluene:BBS-3 solution (Beckman) (3.8 litres of toluene, 18.95 g of PPO, 378.9 mg of POPOP and 100 ml of Bio-Solv BBS-3) was added and mixed. The radioactivity was counted in a liquid scintillation system (Beckman LS-235:counting efficiency for ^{3}H, 70%). Each vial was counted two times in a row for 10 minutes. The second reading was always used for computation. The estradiol-17β level for unknown serum samples,were converted to pg/ml from counts per minute, by the use of a computer program (David, 1974).

The radioimmunoassay was validated for the estimation of estradiol-17β in bovine serum. In order to assess the specificity of the assay, estrone, progesterone and corticosterone standards at concentrations identical to estradiol-17β standards were assayed using estradiol-17β antibody. No binding was observed. Using the same antibody England et al. (1974) also reported low cross reactivity of estrone, estriol, progesterone, corticosterone and other related steroids. Accuracy of the estradiol-17β assay examined throughout the range of 10-320 pg/sample showed no systematic error. The quantity of estradiol-17β estimated by assay showed a close correlation ($r=0.99, b=0.52$) with the expected values.

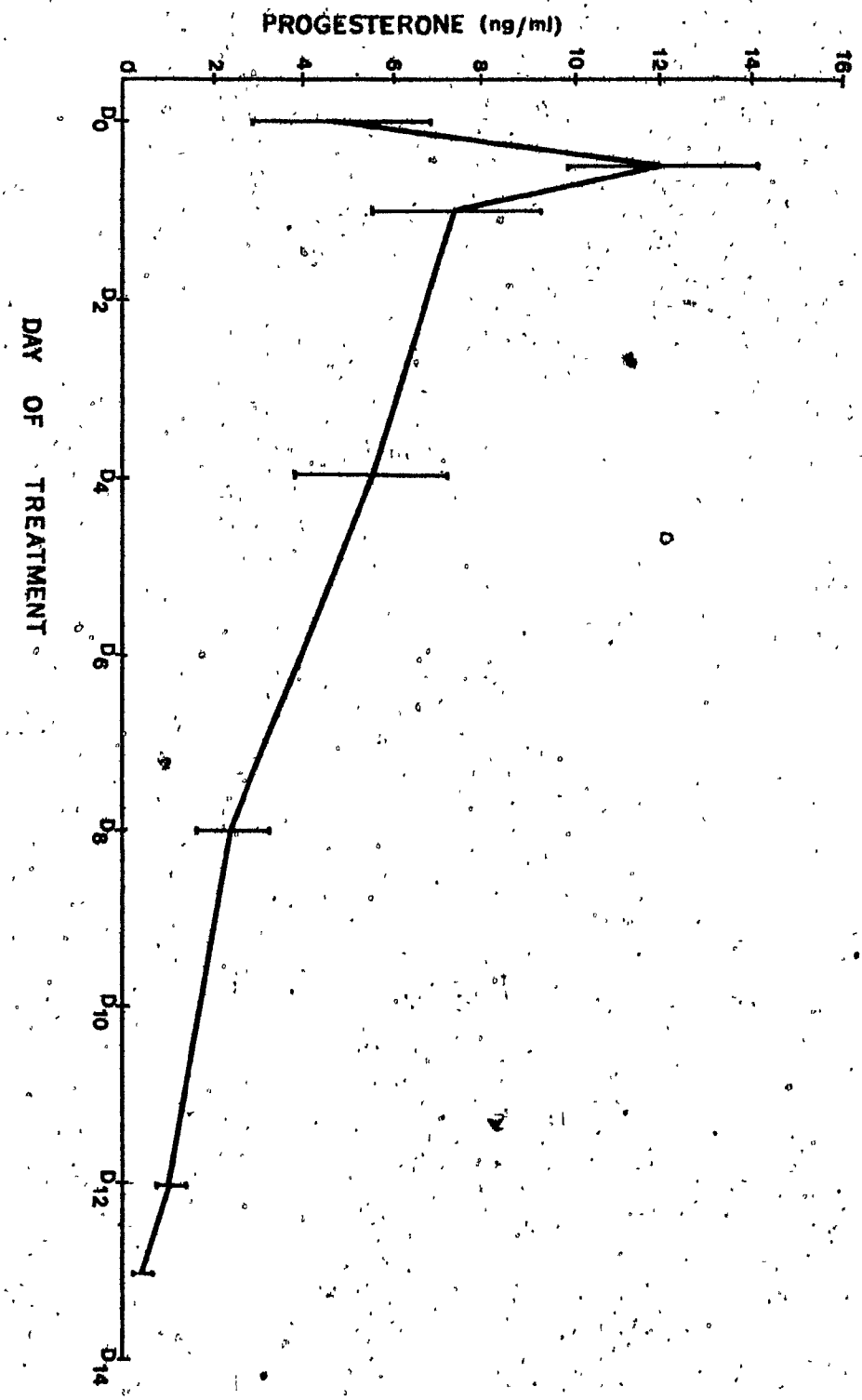
The internal standard (40 pg/sample) included in each assay gave consistent results. The inter- and intra-assay precision (6.8 and 7.2%) were satisfactory for physiological studies. The sensitivity of the method (5 pg/sample) was adequate for analysis of estradiol-17β in 1 ml serum.

Results

Group I

Twenty-one out of 24 heifers retained the device for 12 days. Out of three heifers which lost the vaginal device, two of them were in estrus during the treatment period and had CL at the time of removal. Twenty out of 21 heifers which retained the device were in standing estrus within 48 hours after removal of the device. The mean interval from the removal of the device to the onset of estrus was 30.4 hours. All 20 heifers which exhibited estrus had palpable CL eight days after removal of the device. The heifer which failed to exhibit estrus after removal did not have a palpable CL. The mean concentration of serum progesterone at time zero was 4.8 ng/ml. This was significantly ($P < 0.01$) lower than 12.1 ng observed 12 hours later (Figure 4). After 12 more hours progesterone concentration declined sharply ($P < 0.01$). Thereafter, progesterone declined slowly reaching a mean level 1.1 ng on the day the devices were removed. There was a significant decrease in concentration of

Figure 4. Mean serum progesterone before, during and after insertion of silastic vaginal device containing progesterone in cycling heifers. Two hundred mg of progesterone and 5 mg of estradiol benzoate were also given at D_0. Devices were removed on D_{12}. n = six heifers.


PROGESTERONE (ng/ml)
0
2
4
6
8
10
12
14
16
DAY OF TREATMENT
D0
D2
D4
D6
D8
D10
D12
D14

progesterone 0.6 ng/ml 24 hours after the device was removed. Eighteen heifers returned to estrus approximately 21 days after first treatment estrus.

Group II

Twenty-one out of 24 heifers retained the device until removal (Table 2). The other three heifers, one treated on day 0-4 and the others on day 16-20 of the estrous cycle failed to retain the device for 12 hours. All heifers treated between day 5-15 exhibited estrus within 48 hours of removal of the device. The mean interval from the time the device was removed to the onset of estrus was 42.0 hours. One heifer in each of the remaining groups failed to exhibit estrus. For here the mean interval from removal of the vaginal device to estrus was 39.3 and 36.3 hours respectively for heifers treated on day 0-5 and 16-20. Statistical analysis (t test) revealed no significant ($P > 0.05$) difference in the time of interval to estrus among treatment groups. All heifers which exhibited estrus after device removal had palpable CL eight days after the devices were removed.

Group III

Before inserting the device the mean concentration of serum progesterone was 2.7 ng/ml (Figure 5). Progesterone concentration rose to a maximum of 5.9 ng/ml twelve hours after

Table 2. Effect of stage of cycle at the time of insertion of progesterone + estradiol-17β vaginal device on estrus response and ovulation in cycling heifers.

Parameters	Stage of the Cycle		
	Day 0-4	Day 5-15	Day 16-20
No. treated	8	8	8
Heifers retaining device for 12 days	7	8	6
No. exhibited estrus	6	8	5
Mean interval to estrus in hours	39.3	42.0	36.3
No. ovulated	6	8	5

insertion of the device. A sharp decline ($P < 0.01$) in progesterone concentration was observed on day 2 compared to the day 1 sample (2.8 vs 5.6 ng/ml). Thereafter, the progesterone decreased gradually to 1.3 ng/ml at the time of removal of the device on day 12. Twenty-four hours after removal of the device, there was a significant decrease in progesterone concentration (0.6 ng/ml). The mean concentration of serum estradiol-17β at the time of insertion of the device was 5.9 pg/ml. Mean serum estradiol 12 and 24 hours post-insertion of the device was 29.5 and 27.2 pg/ml, respectively. This concentration was approximately five-fold greater than pretreatment concentration. Estradiol declined to pretreatment concentration (7.4 pg/ml)

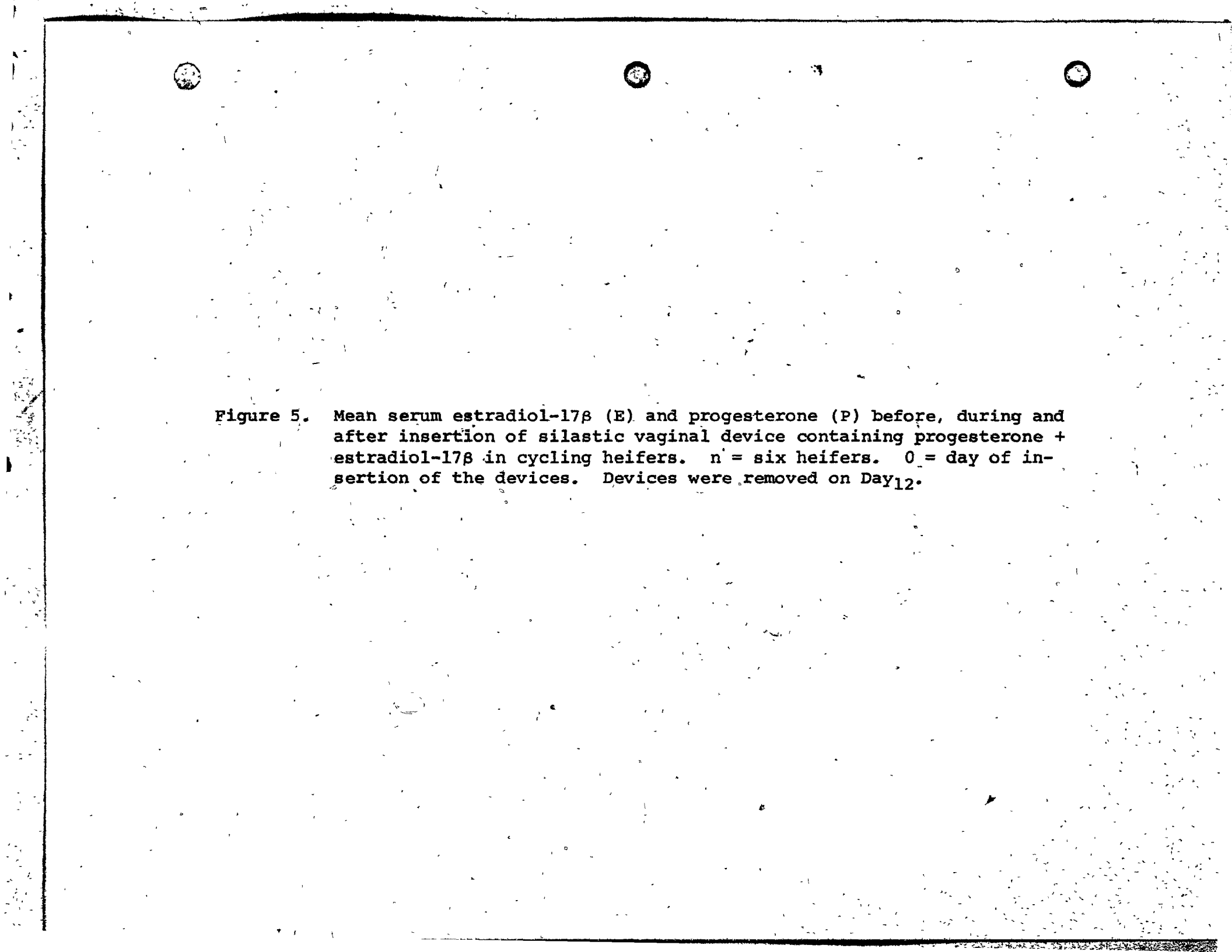
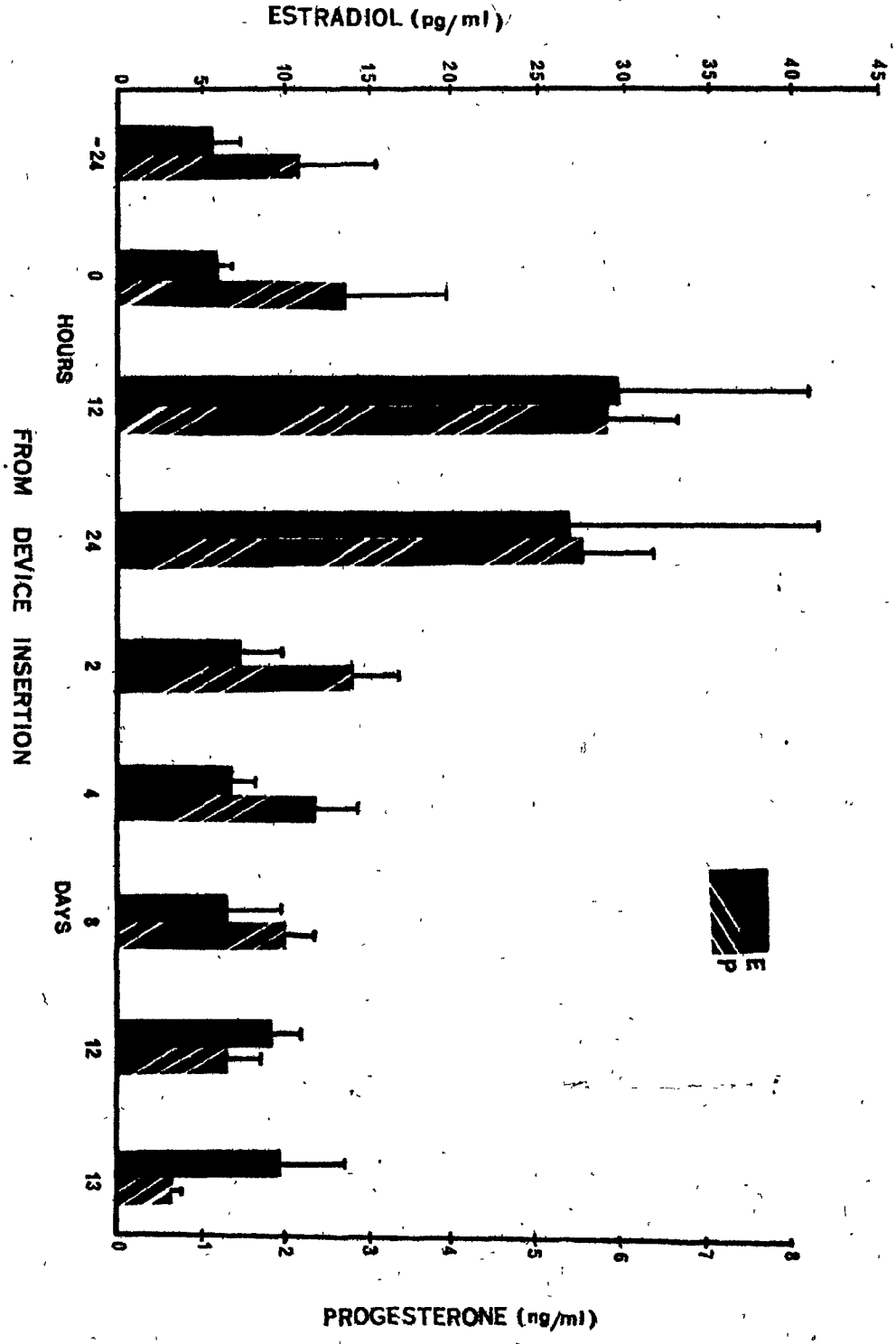


Figure 5. Mean serum estradiol-17β (E) and progesterone (P) before, during and after insertion of silastic vaginal device containing progesterone + estradiol-17β in cycling heifers. n = six heifers. 0 = day of insertion of the devices. Devices were removed on Day_{12}.


ESTRADIOL (pg/ml)
0
5
10
15
20
25
30
35
40
45
-24
0
12
24
2
4
8
12
13
HOURS
DAYS
FROM DEVICE INSERTION
E
P
0
1
2
3
4
5
6
7
8
PROGESTERONE (ng/ml)

days after insertion of the device and remained fairly constant until day 8. A slight increase in serum estradiol was observed at removal of the device on day 12. Nine out of 11 heifers were in standing estrus 36 hours after removal of the device. None of the heifers exhibited estrus during the treatment period. Five of the nine heifers returned to estrus between 17-22 days after the synchronized estrus. Four heifers were diagnosed pregnant by rectal palpation 45 days after insemination. The two heifers which failed to exhibit estrus after device removal (Nos. 277 and 278) had cystic ovaries at the time of palpation 45 days post-insemination.

EXPERIMENT III. EFFECT OF SILASTIC VAGINAL DEVICE CONTAINING ESTRADIOL-17β, PROGESTERONE AND PROGESTERONE + ESTRADIOL-17β ON ESTRUS AND LH RELEASE IN OVARIECTOMIZED HEIFERS

Introduction

Many workers (Hobson and Hansel, 1972; Short et al., 1973; Hausler and Malven, 1976, and others) were able to reproduce the plasma preovulatory LH surge in the ovariectomized cow by the administration of estradiol. An injection of 2 mg of estradiol can induce estrus behaviour and LH surge in prepuberal heifers (Gonzalez-Padilla et al., 1975). These reports suggest that estrogen may trigger an acute release of LH in bovine. Additional support for an estrogen stimulated LH

release can be inferred from plasma estrogen concentration (Ecternkamp and Hansel, 1971; Henricks et al., 1971; Christensen et al., 1971).

The results of the previous experiment and other reports indicate that progesterone will block estrus and ovulation in cattle. The mechanism by which progesterone inhibits estrus and ovulation is not clear in cattle. It has been observed that progesterone will block estrogen mediated LH release in intact ewes (Bolt et al., 1971) and ovariectomized ewes (Scaramuzzi et al., 1971). In support of this mechanism in cattle Hobson and Hansel (1972) observed that estrogen-induced LH release was inhibited in luteal phase heifers. However, others (Short et al., 1973; Hausler and Malven, 1976) failed to inhibit estrogen-induced LH release with progesterone in ovariectomized heifers. In view of the conflicting results further work is necessary to define the mechanism of progesterone action in the control of estrus and ovulation in cattle.

The objectives of this experiment were to determine the effect of silastic vaginal device containing estradiol-17β, progesterone and progesterone + estradiol-17β on estrus response, serum progesterone, estradiol and LH in ovariectomized heifers.

Materials and Methods

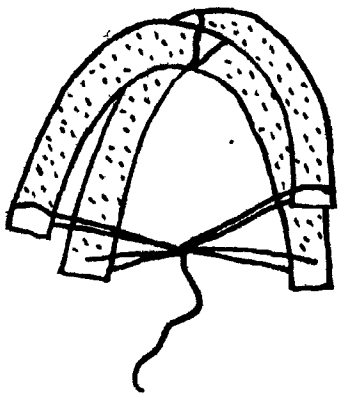
Six heifers 18 to 24 months of age and weighing about 450 kg were ovariectomized about 21 days before the commencement of the experiment. The heifers were housed indoors and were fed on a maintenance ration of grain and hay.

The heifers were assigned randomly to one of three treatments during treatment periods A, B and C. During period A, two heifers were treated with progesterone vaginal device ("P"), two heifers with estradiol-17β vaginal device ("E") and the remaining two heifers with progesterone + estradiol-17β vaginal device ("P+E"). During the period B and C the treatments were altered so that each heifer had the chance to receive all three treatments. The devices were left in the vagina for 12 days during each treatment period. The interval between treatments was seven days.

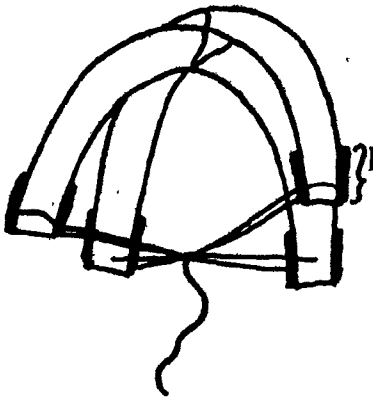
Preparation, insertion and Removal of the Vaginal Devices

Progesterone and progesterone + estradiol-17β vaginal devices were prepared as described in part II. Estradiol-17β vaginal device was made by mixing 10 mg of estradiol-17β with 1 ml of silicone medical adhesive and coating it around the ends of vaginal device described in part II (Figure 6). The approximate surface area of estradiol coating was about 40 cm^2. The three types of devices were autoclaved and weighed before

Figure 6. Diagramatic representation of silastic vaginal devices containing progesterone, estradiol-17β and progesterone + estradiol-17β.

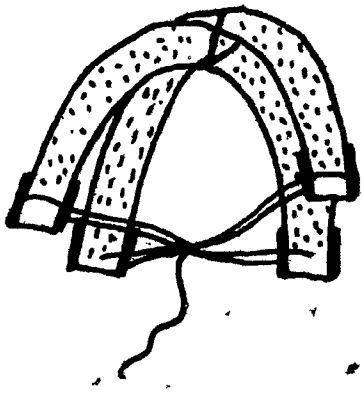


PROGESTERONE VAGINAL DEVICE



ESTRADIOL VAGINAL DEVICE

ESTRADIOL COATING



PROGESTERONE+ESTRADIOL VAGINAL DEVICE

insertion. The devices were inserted into the vagina with the help of a plastic speculum and plunger. Twelve days after insertion the devices were removed by pulling on a string attached to the device and left protruding from the vulva. Following removal, devices were cleaned, dried and weighed.

Blood Samples and Estrus Detection

Blood samples (20 ml) were obtained from the jugular vein before insertion at -24 hr, 0 hr, and at 4 hr intervals from the time of insertion to 36 hours post-insertion of the vaginal device. Thereafter, blood samples were obtained on days 2, 4, 8, 12 and 24 hours post-device removal. The serum was transferred in small aliquots and stored in the freezer at -20°C until assay.

The heifers were observed closely for signs of estrus at each bleeding period and throughout the experimental period. A vasectomized bull and Kamar heat mount detectors were used to assess the onset of standing estrus.

Serum Progesterone, Estradiol-17β and LH Analyses

Serum progesterone and estradiol-17β were measured by the procedures described in parts I and II. Dr. Gordon D. Nisewender of Colorado State University, Fort Collins, Colorado, kindly analyzed the serum samples for LH.

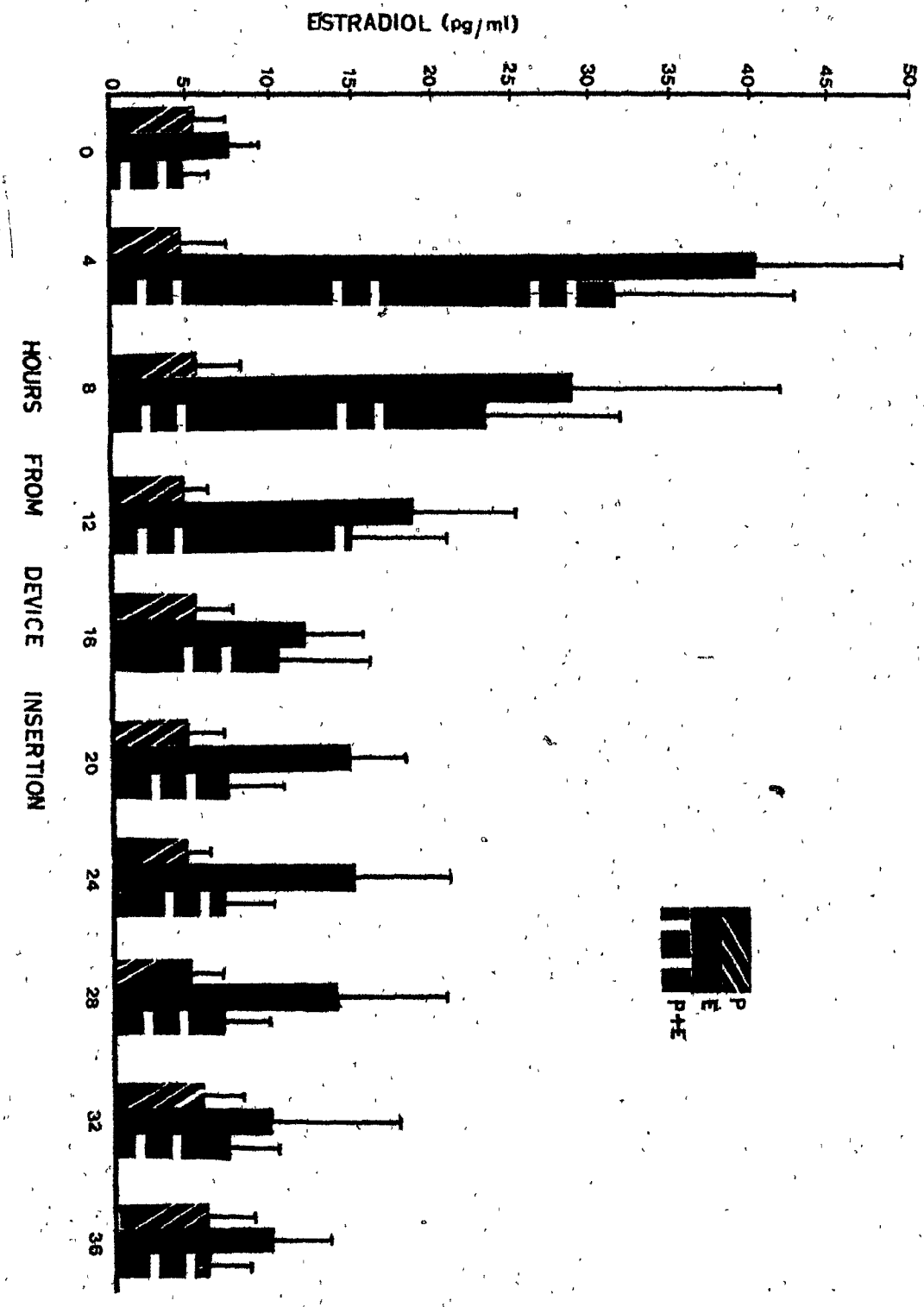
Statistical Analyses

Serum hormone data were analyzed statistically as a split split plot experiment in a completely randomized design. Periods were considered as main plot unit. Blood sampling times were considered as subplot units and treatments as sub sub plot units. The means were analyzed using LSD (Steel and Torrie, 1960).

Results

The mean estradiol-17β concentration in serum collected 24 hours before and just prior (0 hr) to insertion of steroid vaginal device was not different among treatment groups (Figure 7). The mean serum estradiol remained relatively constant throughout the sampling period in "P" treated heifers (5.1 pg/ml). Serum estradiol, four hours after insertion of vaginal device, was about 40.9 and 32.1 pg/ml for "E" and "P+E" treated heifers, respectively. These concentrations were greater than ($P < 0.01$) pretreatment concentration and all concentrations for "P" treated heifers. After serum estradiol in "E" treated heifers reached a maximum concentration, estradiol-17β declined steadily and reached 12 pg/ml 16 hours after insertion of the vaginal device. Thereafter, the mean estradiol in these heifers remained constant for the remainder of the sampling period. In two of these "E" treated heifers serum estradiol declined to pretreatment level 28 hours after insertion of the device. In

Figure 7. Mean serum estradiol-17β in ovariectomized heifers treated with vaginal device containing progesterone (P), estradiol-17β (E) or progesterone + estradiol-17β (P+E). 0 = time of insertion of the devices. n = six observations per treatment. Analysis of variance of serum estradiol-17β showed that estradiol concentration varied with treatment. Highest concentration was observed in heifers treated with E device and which was significantly different from that observed with P ($P < 0.01$) and P+E ($P < 0.05$). Values for the rest of the sampling period appear in Appendix Table 5.

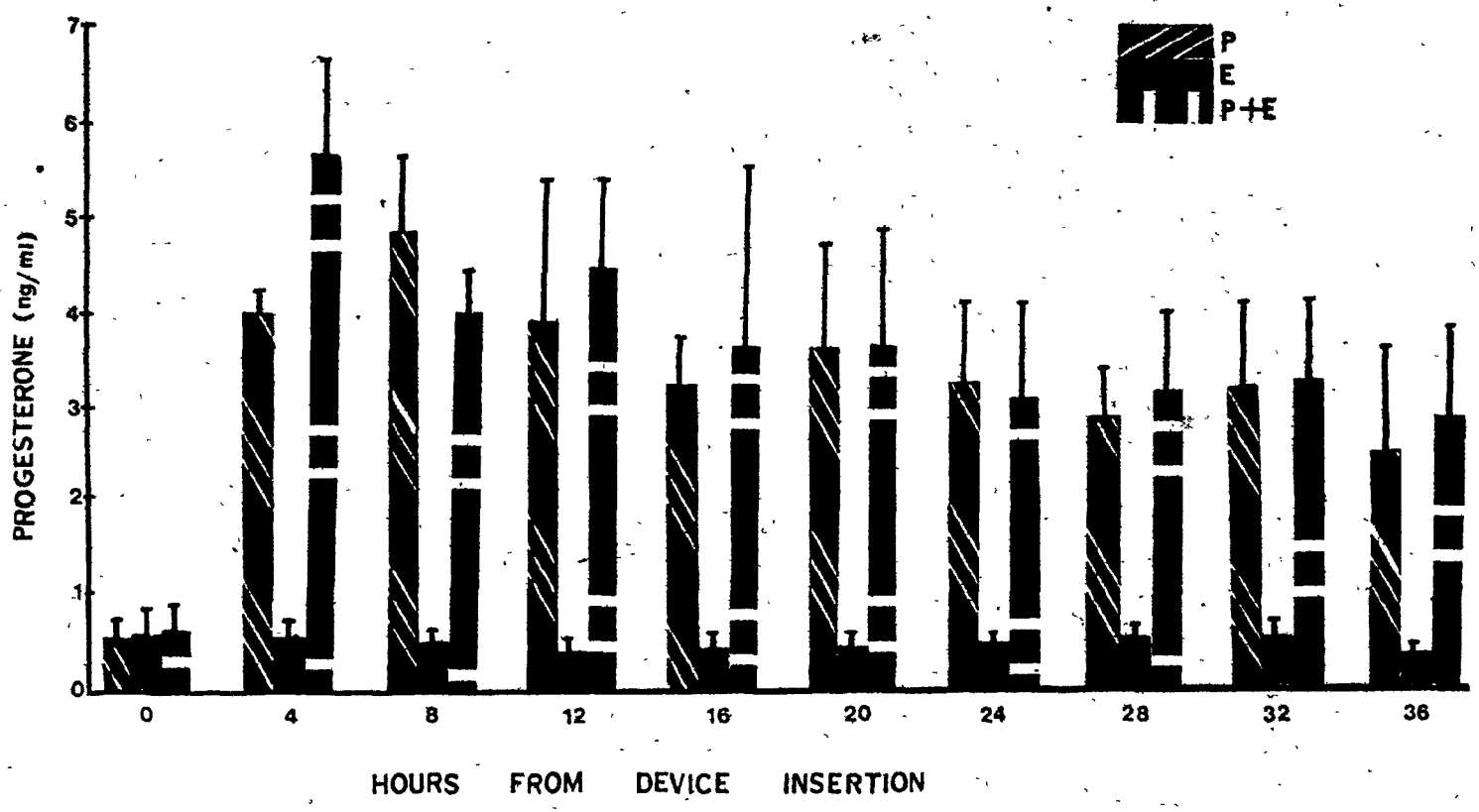

ESTRADIOL (pg/ml)
0
5
10
15
20
25
30
35
40
45
50
HOURS FROM DEVICE INSERTION
0
4
8
12
16
20
24
28
32
36
P
E
P+E

"P+E" treated heifers the maximum concentration of estradiol-17β occurred at 4 hours and declined significantly ($P < 0.01$) to 7.4 pg/ml at 20 hours and thereafter estradiol concentration remained constant.

Mean progesterone 24 hours before and just prior (0 hr) to the insertion of the device was not different among treatment groups (Figure 8). Serum progesterone of heifers treated with "E" device remained at about 0.5 ng/ml throughout the sampling period. The mean serum progesterone four hours after insertion of "P" device was increased ($P < 0.01$) relative to comparable mean for "E" treated heifers, 3.9 vs 0.5 ng/ml. The maximum concentration of serum progesterone occurred in "P" treated heifers eight hours after insertion of the device. Thereafter the concentration declined gradually during the sampling period and reached 1.3 ng/ml at device removal on day 12. A significant ($P < 0.01$) decline in serum progesterone was observed in "P" treated heifers 24 hours after removing the device. The mean serum progesterone in "P+E" treated heifers showed a similar pattern as in "P" treatment. However, the maximum progesterone concentration in "P+E" treated heifers was observed four hours after insertion of the device.

Pretreatment LH concentration ranged from 5-6 ng/ml. In heifers treated with "P" and "P+E" no change was observed in serum LH during the sampling period (Figure 9). However, a

Figure 8. Mean serum progesterone in ovariectomized heifers treated with vaginal device containing progesterone (P), estradiol-17β (E) or progesterone + estradiol-17β (P+E). 0 = time of insertion of the devices. n = six observations per treatment. Analysis of progesterone data showed progesterone in serum was similar ($P > 0.05$) following treatment with silastic vaginal device containing P or P+E. Values for the rest of the sampling period appear in Appendix Table 6.


P
E
P+E
PROGESTERONE (ng/ml)
0
1
2
3
4
5
6
7
0
4
8
12
16
20
24
28
32
36
HOURS FROM DEVICE INSERTION

slight ($P > 0.05$) decline in LH concentration was observed at four and eight hours post device insertion in heifers treated with "P+E" and "P", respectively. In "E" treated heifers also a slight decline ($P > 0.05$) in serum LH was observed four hours after insertion of the device. Thereafter in "E" treated heifers serum LH increased and reached a maximum concentration of 32.7 ng/ml 16 hours after insertion of the device. After the maximum concentration a significant ($P < 0.01$) decline in serum LH was observed 24 hours after inserting the device. Four heifers in "E" treatment exhibited another maximum concentration on day 2 and day 4 after device insertion. The serum LH in "E" treated heifers declined to pretreatment concentration eight days after insertion of the device.

Analysis of variance of serum hormone data showed that period did not affect the response. Analysis revealed a significant treatment effect on levels of serum estradiol and LSD showed that the release of estradiol from estradiol-17β device is higher ($P < 0.01$) than that released by "P+E" device. Analysis of variance of estradiol concentration also showed a significant ($P < 0.01$) treatment x time interaction. Analysis of progesterone data showed that progesterone in serum was similar ($P > 0.05$) following treatment with "P" and "P+E" device. However, analysis showed a treatment x time interaction ($P < 0.01$). Serum progesterone in heifers treated with "P+E" was greater

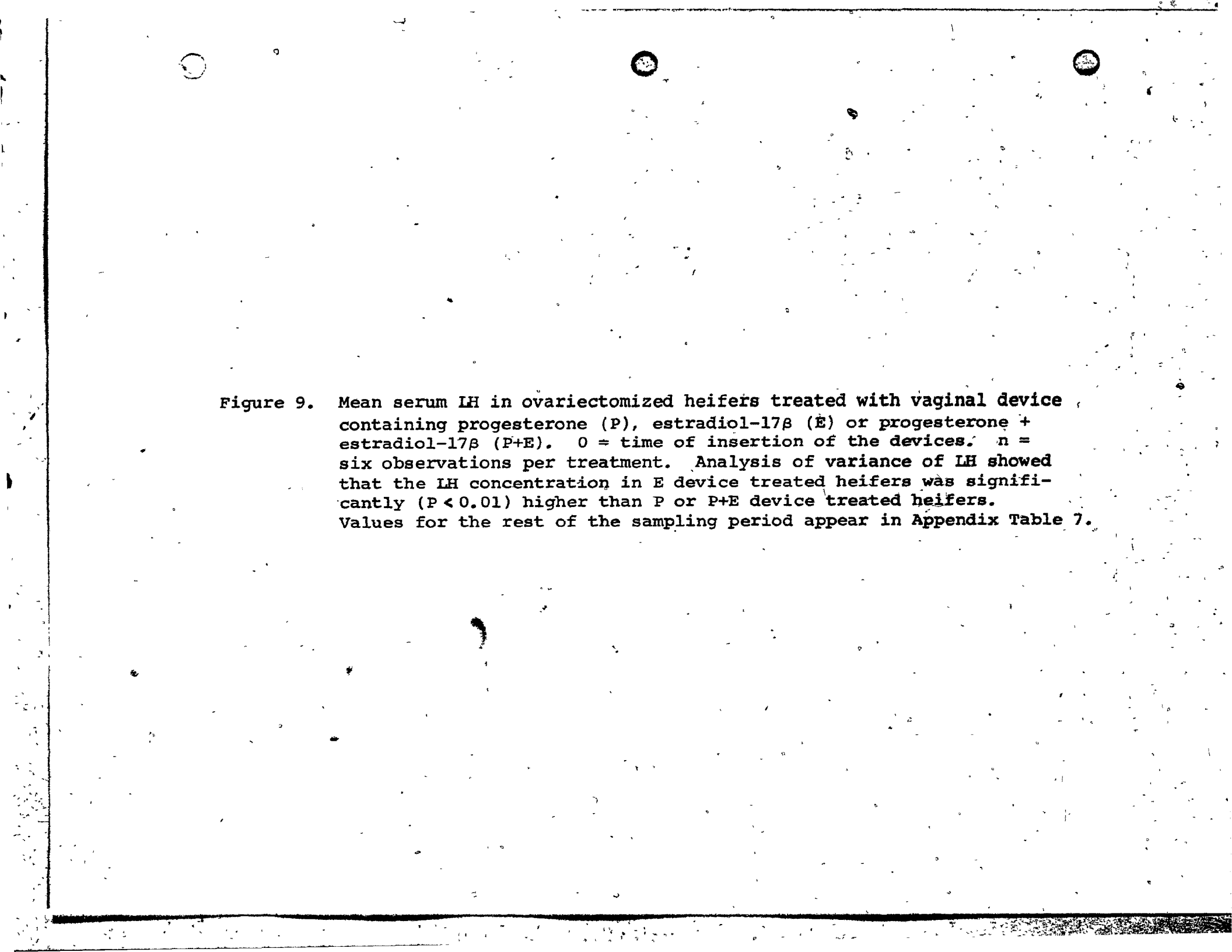
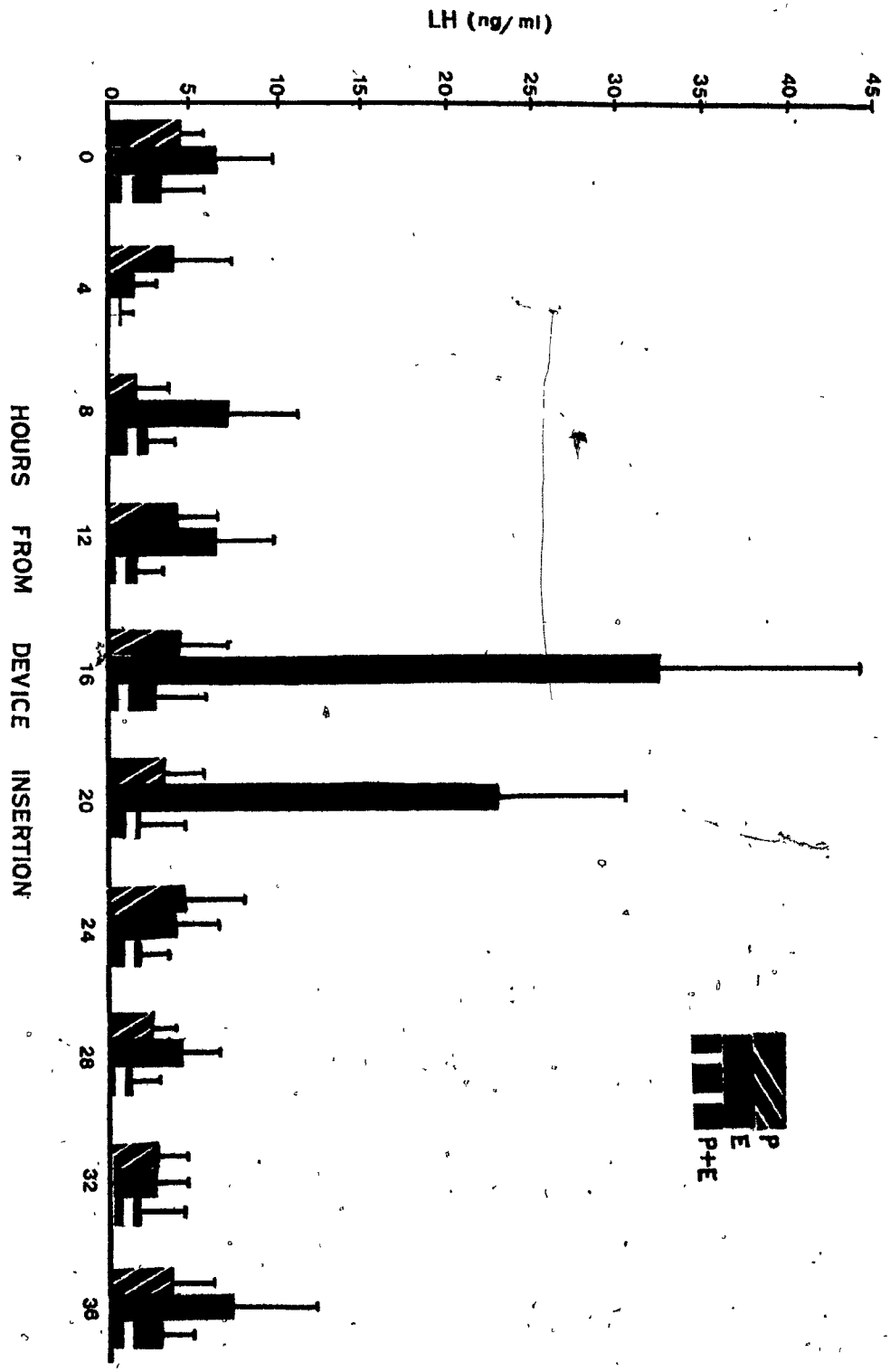


Figure 9. Mean serum LH in ovariectomized heifers treated with vaginal device containing progesterone (P), estradiol-17β (E) or progesterone + estradiol-17β (P+E). 0 = time of insertion of the devices. n = six observations per treatment. Analysis of variance of LH showed that the LH concentration in E device treated heifers was significantly ($P < 0.01$) higher than P or P+E device treated heifers. Values for the rest of the sampling period appear in Appendix Table 7.


LH (ng/ml)
0
5
10
15
20
25
30
35
40
45
0
4
8
12
16
20
24
28
32
36
HOURS FROM DEVICE INSERTION
P
E
P+E

($P < 0.05$) at four hours after insertion of the device and lower ($P < 0.05$) on days 4, 8 and 12 of the sampling period compared to heifers treated with "P" device. Analysis of variance of LH concentration revealed that the LH concentration in "E" treated heifers was significantly higher ($P < 0.01$) than for the "P" and "P+E" treated heifers. No difference ($P \geq 0.05$) in LH concentration was observed between "P" and "P+E" treated heifers.

All heifers treated with "E" device exhibited standing estrus. The onset of estrus from the time of insertion of the device ranged from 18 to 26 hours. Two heifers treated during period A had shorter interval to estrus compared to other heifers. Two of the "P+E" treated heifers also exhibited slight manifestation of estrus 24 hours after device insertion during period A.

All heifers retained the vaginal device for 12 days except for one heifer that expelled the device on day 8 and 10, during period C. The device was cleaned and reinserted within half an hour after expulsion. A whitish yellow vaginal mucus discharge was observed during the treatment period in 25% of the heifers. When the devices were removed they were coated with a thick whitish yellow mucus. The amount of steroid released from the devices was estimated by weighing the devices before and after insertion. No difference in weight was observed in "E" device. A difference in weight of approximately 0.5 g

was observed for "P" and "P+E" device. This indicates that an average of 0.5 g of steroid was released during the 12-day period by both "P" and "P+E" devices.

EXPERIMENT IV. THE EFFECT OF SILASTIC VAGINAL DEVICE CONTAINING PROGESTERONE AND PROGESTERONE + ESTRADIOL-17β ON THE PRESENCE OF CYTOPLASMIC ESTROGEN AND PROGESTERONE RECEPTORS IN TARGET ORGANS

Introduction

Studies on the induction of puberty and the blood hormone concentration profile at the onset of natural puberty in prepuberal heifers suggest that progesterone may be involved in the final events leading to puberty. In determining the value of progesterone as an aid in inducing estrus and ovulation in anestrous ewes, Robinson (1959) indicated that progesterone conditioned receptors in higher centers to respond to low levels of estrogen. One possible way by which progesterone may influence the final events leading to puberty in heifers is as follows. Progesterone priming may increase the cytoplasmic estrogen receptors at the anterior pituitary and/or hypothalamus. This in turn will increase the sensitivity of the receptors for blood estradiol, resulting in depletion of cytoplasmic estradiol receptors, which in turn initiates gonadotrophin surge and ovulation.

The objectives of this experiment were to study the

influence of silastic vaginal device containing either progesterone, progesterone + estradiol-17β or no steroid on serum hormone levels and the presence of cytoplasmic estrogen and progesterone receptors in the hypothalamus, anterior pituitary and reproductive organs of the prepuberal heifer.

Materials and Methods

Eleven prepuberal Hereford-Holstein heifers, 18-21 weeks of age and weighing approximately 135-200 kg, were used. They were housed indoors and fed on a maintenance ration of grain and hay. They were palpated rectally prior to the commencement of treatments and none were cycling.

The heifers were allocated at random to the following treatment groups. Three heifers were treated with silastic vaginal device containing no steroid and were designated as controls ("C"). Four heifers were treated with silastic vaginal device containing 2 g of progesterone ("P"). Four heifers were treated with silastic vaginal device containing 2 g of progesterone and 10 mg of estradiol-17β ("P+E"). Five heifers, one from "C" and two each from "P" and "P+E" were slaughtered 48 hours after insertion of the device. In the remaining six heifers, the device was left in place for a period of 12 days; they were slaughtered 48 hours after removal of

the device. Immediately after slaughter, the uterus, ovaries, adrenals, anterior pituitary, anterior hypothalamus, posterior hypothalamus, portion of cortex tissue, portion of mammary tissue and portion of liver were removed, weighed and placed on ice in a 4°C room.

Vaginal Devices

Silastic vaginal devices containing progesterone and progesterone + estradiol-17β were prepared as described in part II. Control device had the same surface area as others without any steroid in it.

Blood Samples and Estrus Detection

Blood samples (20 ml) from the jugular vein were obtained at -24, 0, 6, 12, 18, 24, 30, 36, 48 hours and 2, 4, 8, 12 days after insertion of the device (0 = time of insertion). Other blood samples were taken 6, 12, 24 and 48 hours after removal of the device. Serum samples were stored in aliquots at -20°C until assay. Serum progesterone and estradiol-17β were determined as described in parts I and II. Serum LH was kindly assayed by Dr. G. Nisewender of Colorado State University, Fort Collins, Colorado.

Heifers were observed for signs of estrus during the treatment period, with an aid of a vasectomized bull and Kamar heat mount detectors.

Steroid Receptor Assay

The steroid binding assay described by Korenman (1975) was used with slight modifications.

Chemicals

a - Steroids

1. Estradiol-17β-2,4,6,7-^{3}H (NEN)
   Specific activity 110 Curies/mM

2. Estradiol-17β (Sigma)

3. Progesterone-1,2-^{3}H (NEN)
   Specific activity 47.8 Curies/mM

4. Progesterone (Sigma)

5. 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione (R 5020) ^{3}H (Roussel UCLAF)
   Specific activity 51.4 Curies/mM

6. R 5020 (Roussel UCLAF)

b - Chemicals for Buffer Solutions

1. Tris (Anachemia Chemical Ltd.)

2. EDTA (Anachemia Chemical Ltd.)

3. 1 N HCl (Baker and Adamson)

4. Dithiothreitol (DTT) (Sigma)

5. Charcoal (NORITA) (J.T. Baker Chemical Co.)

6. Dextran (DEX) (NBCo.)

c - Scintillation solution components

1. Toluene, analytical grade (Mallinckrodt)

2. PPO, scintillation grade (Packard Instrument Co.)

3. POPOP, scintillation grade (Packard Instrument Co.)

4. Bio-Solv BBS-3 (Beckman Instruments, Inc.)

Solutions

a - TED BUFFER - 10 mM (TRIS), 1.5 mM (EDTA), 0.5 mM (DTT), pH 7.4. This was used for the preparation of homogenate and for receptor assay.

b - DEXTRAN COATED CHARCOAL Buffer (DCC) - 10 mM (TRIS), 250 mg (NORIT), 2.5 mg (DEX). The pH was adjusted to 8.0 with HCl and the volume made to 100 ml. This buffer was used to separate the bound from unbound steroid.

c - Scintillation counting solution - 3.8 litres of toluene, 18.95 g (PP)), 378.9 mg (POPOP) and 100 ml Bio-Solv BBS-3.

Preparation of the Cytosol

Immediately after recovery of the tissues, they were individually trimmed of connective tissue, minced and homogenized (1 g of tissue in 5 ml of TED Buffer) using Polytron (Brinkmann) at full speed for one minute. After four hours at 4°C, the homogenate was centrifuged at 105,000 g for one hour at 4°C in an ultracentrifuge (Beckman L2-65B). The concentration of protein in the supernatant was determined (Lowry et al., 1951) and 1-2 ml aliquots of the supernatant were stored in liquid N_2 or at -20°C until receptor assay.

Binding Assay

The incubation generally followed the design shown in Table 3. A total volume of 0.4 ml contained 0.1 ml TED buffer, 0.1 ml labelled steroid and 0.2 ml of cytosol (homogenate). In the blanks, the cytosol was replaced by the same volume of

Table 3. Estradiol and progesterone protein binding procedure. Assay design.

Blank
100 µl TED Buffer
100 µl*
200 µl TED Buffer
Maximal Binding
100 µl TED
100 µl*
200 µl homogenate
Displacement by excess unlabelled steroid
100 µl of excess unlabelled steroid
100 µl*
200 µl homogenate
*Concentration Molar of labelled steroid $1x10^{-11}, 1x10^{-10}, 1x10^{-9}, 1x10^{-8}, 2x10^{-8}, 4x10^{-8}$,

TED buffer. In the incubations where the displacement of labelled steroid from receptor sites was measured the buffer contained an excess of cold steroid. All incubations were done in duplicate. The homogenate was thawed and adjusted with TED buffer to a final concentration of 2 mg of protein/ml of homogenate. After adding the homogenate, the assay tubes were vortexed for 10 seconds and incubated for about 18 hours. After incubation, 0.5 ml of freshly prepared dextran-coated charcoal buffer (DCC) was added; the tubes were vortexed for 10 seconds and allowed to stand at 4°C for 30 minutes. The tubes were then centrifuged at 2,000 g for 10 minutes. The supernatant was

decanted into vials containing 10 ml of scintillation solution. The vials were shaken and left in the dark for 24 hours before counting.

Specific steroid binding for six levels of labelled steroid added was calculated by subtracting the counts in blanks and those containing an excess of cold steroid from each determination. Total radioactivity present in the incubation was estimated by omitting the charcoal. Counts per minute were then converted to pico moles of labelled steroid bound/mg of protein. Pico moles labelled steroid bound/mg protein and molar concentration of labelled steroid added were then plotted on a semi-logarithmic graph paper and the pico moles of labelled steroid bound at saturation point was determined.

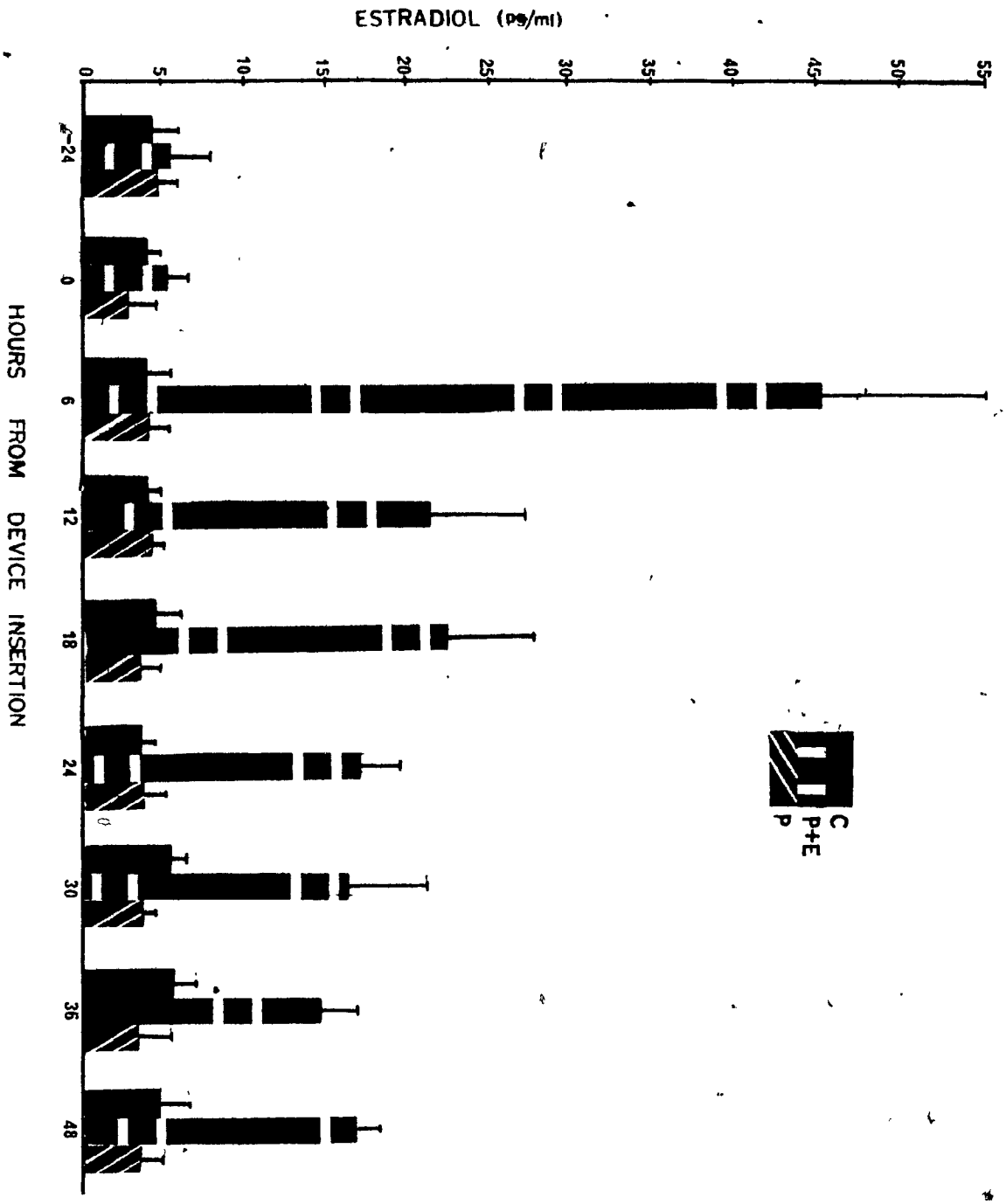
Statistical Analyses

The concentration of hormones, the weights of the heifer, the weights of adrenals, ovaries, uterus and anterior pituitary, and steroid receptor concentrations were analyzed by regression, as a split split plot experiment in a completely randomized design.

Results

Mean estradiol-17β and progesterone concentration in serum collected just prior to vaginal device treatment were not different ($P > 0.05$) among treatment groups (Figure 10). Serum estradiol-17β four hours after insertion of the device averaged 45.1 pg/ml

Figure 10. Mean serum estradiol-17β in prepuberal heifers treated with vaginal device containing no steroid (C), progesterone + estradiol-17β (P+E) or progesterone (P). 0 = time of insertion of the devices. n = three heifers for group C and four heifers each for groups P+E and P. Values for the rest of the sampling period appear in Appendix Table 8.


ESTRADIOL (pg/ml)
0
5
10
15
20
25
30
35
40
45
50
55
-24
0
6
12
18
24
30
36
48
HOURS FROM DEVICE INSERTION
C
P+E
P

in "P+E" treated heifers. Subsequent to this maximum, estradiol concentration decreased significantly ($P < 0.01$) eight hours post device insertion and, thereafter, the concentration decreased slowly. Forty-eight hours after insertion of the device, the serum estradiol concentration averaged 16.5 pg/ml. Serum estradiol returned to basal level on day 4 of treatment. No significant change ($P > 0.05$) in serum estradiol concentration was observed in heifers treated with "P" or "C" devices.

The concentration of serum progesterone was 4.2 ng/ml four hours after insertion of the device in both "P" and "P+L" treated heifers (Figure 11). After reaching the maximal value, the serum progesterone in "P" and "P+L" treated heifers decreased respectively to 2.5 and 1.5 ng/ml at device removal. A significant decrease ($P < 0.01$) in serum progesterone was observed in "P" and "P+E" treated heifers after removal of the device. No change in serum progesterone was observed in control heifers.

The serum LH, quantitated in the six heifers treated with vaginal device for 12 days, was generally very low or non detectable during the treatment period. No change in serum LH was observed in progesterone treated heifers before, during and after insertion of the device (Table 4). In "P+E" treated heifers, one heifer had a maximum concentration of 7.6 ng/ml 12 hours post device removal and the other had 16.6 ng/ml 24

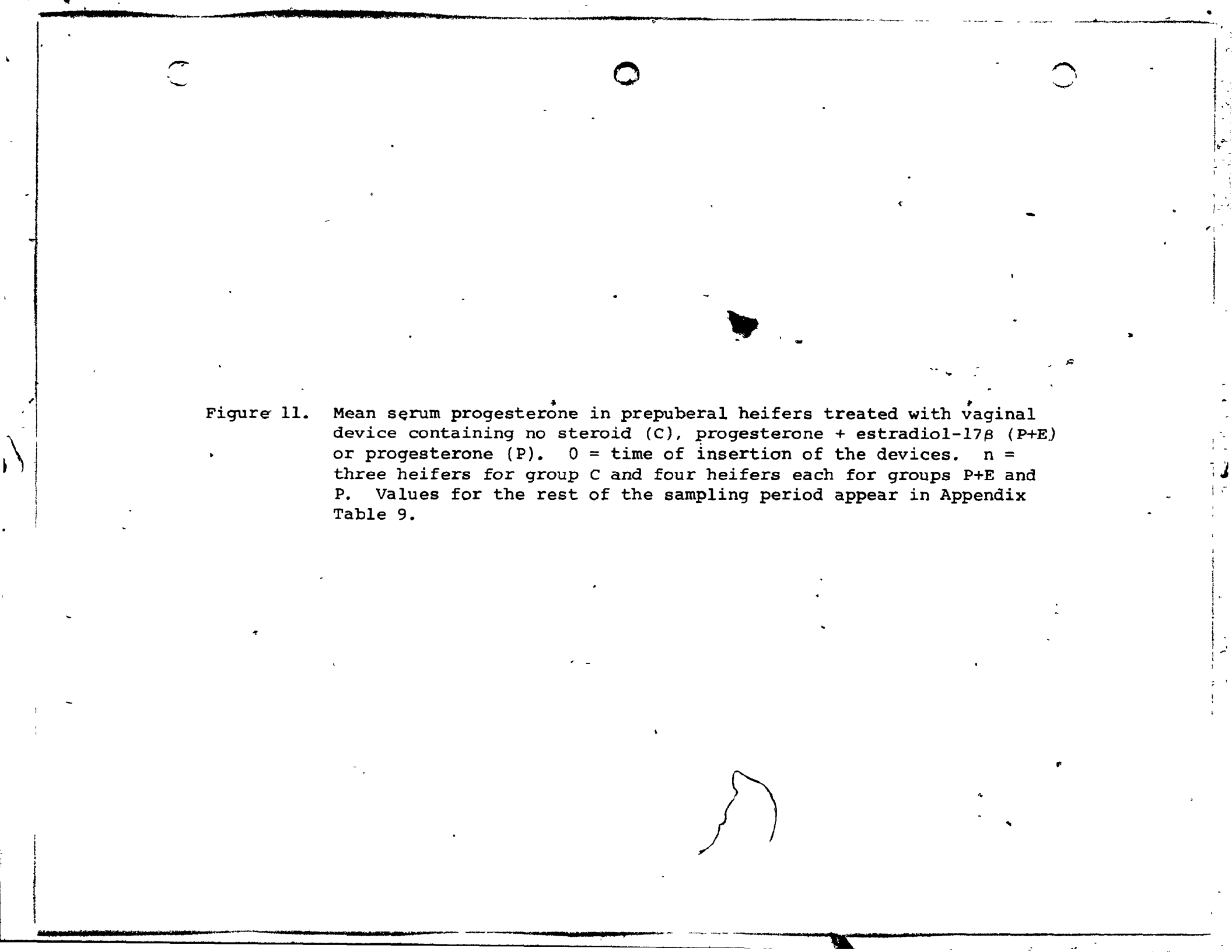


Figure 11. Mean serum progesterone in prepuberal heifers treated with vaginal device containing no steroid (C), progesterone + estradiol-17β (P+E) or progesterone (P). 0 = time of insertion of the devices. n = three heifers for group C and four heifers each for groups P+E and P. Values for the rest of the sampling period appear in Appendix Table 9.

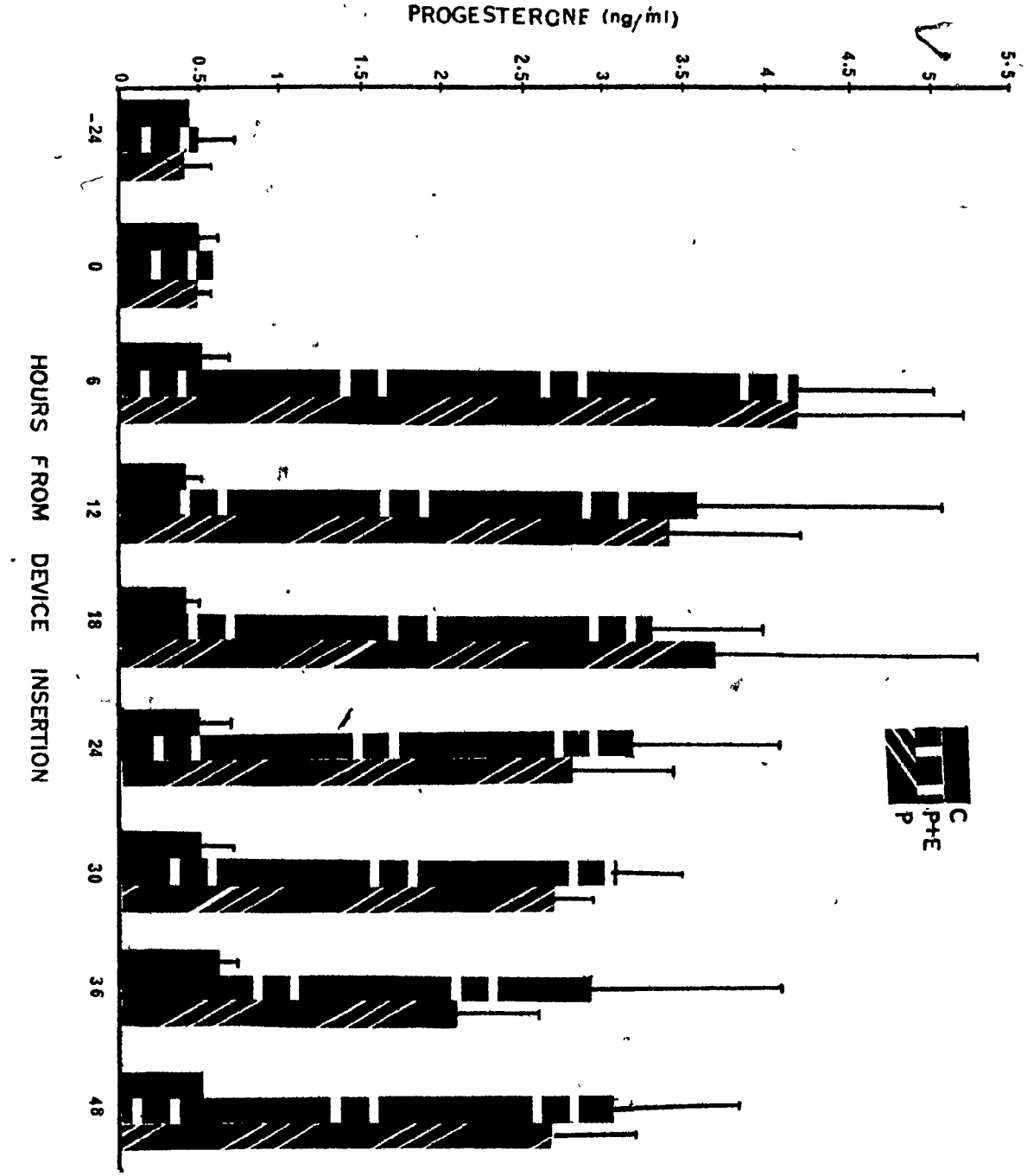

PROGESTERONE (ng/ml)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
HOURS FROM DEVICE INSERTION
-24
0
6
12
18
24
30
36
48
C
P+E
P

Table 4. Variability in serum LH concentration** in prepuberal heifers treated with control, progesterone and progesterone + estradiol-17β silastic vaginal device.

Time	Progesterone		Progesterone+Estradiol		Control	
	15*	26	10	29	16	31
-24 hr	9.97	0.47	0.94	0.25	2.00	4.00
0 hr	1.19	0.47	1.77	0.71	0.66	8.56
6 hr	0.26	0.40	1.02	0.31	0.57	2.07
12 hr	ND	0.48	0.31	1.00	0.89	3.22
18 hr	ND	0.20	ND	0.94	0.66	26.40
24 hr	ND	0.22	0.51	0.49	0.41	2.25
30 hr	ND	0.29	ND	1.71	0.31	1.68
36 hr	ND	ND	ND	1.24	0.15	3.21
48 hr	0.58	ND	ND	1.17	0.12	3.20
D_4	ND	ND	0.37	0.53	ND	3.58
D_8	0.63	ND	0.16	0.95	ND	52.21
D_{12}, 0 hr	0.40	ND	ND	1.97	0.16	14.91
D_{12}, 6 hr	1.30	2.4	0.60	1.64	0.15	1.40
D_{12}, 12 hr	1.51	0.46	7.65	2.05	0.04	2.10
D_{12}, 18 hr	1.46	2.49	0.45	2.14	ND	1.94
D_{13}	0.81	0.98	0.26	16.56	0.34	32.95
D_{14}	2.86	1.49	0.81	1.20	0.72	1.65

*Animal number.
**ng/ml
0 hr = Time of device insertion.

D_{12}, 0 hr = Time of device removal.

hours after the device removal. In one "C" treated heifer, LH peaks were observed at 18 hours, on day 8, at device removal and 24 hours after removal of the device.

The analysis of regression on body weight revealed that there was no difference ($P > 0.05$) in the body weight among treatment groups. Hence no adjustment of organ weights was made in

relation to body weight. The analysis also showed that the duration of the steroid device treatment did not affect organ weights ($P > 0.05$). The analysis of organ weights revealed that uterine weight was affected by treatment (Table 5). "P" and "P+E" treated heifers had signficiantly ($P < 0.01$) higher uterine weights (70.9 and 73.8 g, respectively) than control heifers (48.3 g). Ovarian weight was also affected by treatment ($P < 0.05$). Ovarian weight in "C" treated heifers (4.7 g) was higher than in "P" and "P+E" treated heifers. Ovarian weight for "P" treated heifers was greater ($P < 0.05$) than for "P+E" treated heifers. The mean weight of pituitary was lower ($P < 0.05$ in "P" treated heifers than in "C" and "P+E" treated heifers. A positive correlation ($P < 0.05$) was obtained between (a) uterine and adrenal weight (0.54), (b) uterine and anterior pituitary weight (0.31), (c) ovarian and adrenal weight (0.41), and (d) ovarian and anterior pituitary weight (0.52).

The highest concentration (5.8 pico moles/mg cytosol protein) of uterine estrogen receptors were observed in "C" treated heifers (Table 6). This was significantly different ($P < 0.05$) from that of "P" and "P+E" device treated heifers. Indeed, "P+E" treated heifers had the lowest concentration (2.1 pico moles/mg cytosol protein), which was significantly different ($P < 0.05$) from "C" and "P" treated heifers. The progesterone receptor concentration in uterus, when tritiated progesterone was used for binding,

Table 5. Effect of progesterone and progesterone + estradiol-17β device treatment on uterine, ovarian, adrenal and anterior pituitary weights in prepuberal heifers*.

Treatment Groups	No. of Animals	Body Wt (kg)	Uterine Wt (g)	Ovarian Wt (g)	Adrenal Wt (g)	A. Pit. Wt (g)
Control	3	172[a] (161-193)	48.3[a] (40.1-59.5)	4.7[a] (2.0-8.7)	7.6[a] (6.6-8.6)	0.92[a] (0.89-0.96)
Progesterone Device	4	168[a] (147-189)	70.9[b] (40.4-122.2)	3.9[b] (3.3-5.3)	8.5[a] (86.2-10.6)	0.94[b] (0.87-1.01)
Progesterone + Estradiol-17β Device	4	164[a] (136-177)	73.8[b] (54.6-95.4)	2.9[c] (1.7-3.4)	7.9[a] (6.9-8.6)	0.92[a] (0.79-1.01)

*Average figures with ranges in parenthesis.

[a,b,c]Observations bearing superscript indicate the differences and similarities in columns. Significant level $P < 0.05$.

Table 6. Effect of progesterone and progesterone + estradiol-17β vaginal device treatment on the presence of estrogen and progesterone receptor sites* in the uterus of prepuberal heifers.

Treatment	Estrogen Receptors	Progesterone Receptors	
	^{3}H-E$_2$ Bound	^{3}H-P Bound	^{3}H-R5020 Bound
Control (3)	5.87^a ±0.40	1.56^a ±0.28	2.71^a ±0.91
Progesterone (4)	3.51^b ±1.50	0.73^b ±0.44	1.49^a ±0.91
Progesterone + Estradiol-17β (4)	2.08^c ±0.98	1.02^b ±0.26	2.16^a ±0.78

*Pico moles of steroid bound at saturation point/mg protein of tissue homogenate.

a, b, c Observations bearing superscript indicate the differences and similarities in columns. Significant $P < 0.05$.

( ) = No. of animals.

was highest (1.6 pico moles/mg cytosol protein) in "C" treated heifers which was significantly different ($P < 0.05$) from other two treated groups. The binding of tritiated R5020 to uterine progesterone receptors was twice as high ($P < 0.01$) as that of tritiated progesterone. However, no difference ($P > 0.05$) was observed in uterine progesterone receptor concentration among treatment groups. There was no significant ($P > 0.05$)

effect of treatment on estrogen and progesterone receptors in the posterior hypothalamus and the anterior pituitary (Tables 7 and 8). However, "P" and "P+E" heifers had slightly higher estrogen and progesterone receptors than "C" treated heifers. The mean concentration of estrogen receptor in anterior hypothalamic tissue of "P" and "P+E" treated heifers was slightly higher ($P > 0.05$) than in control heifers (Table 9). The progesterone receptors were not detectable in anterior hypothalamic tissue. Estrogen and progesterone receptors of ovary, liver, adrenal, cerebral cortex, and mammary were not detectable by the present procedure. The analysis of regression showed that time of slaughter did not have any effect ($P > 0.05$) on estrogen and progesterone receptors in uterine, anterior pituitary and anterior and posterior hypothalamic tissues.

Table 7. Effect of progesterone and progesterone + estradiol-17β vaginal device treatment on the presence of estrogen and progesterone receptor sites* in the posterior hypothalamus of prepuberal heifers.

Treatment	Estrogen Receptors	Progesterone Receptors	
	^{3}H-E2 Bound	^{3}H-P Bound	^{3}H-R5020 Bound
Control (3)	0.1120 ±0.008	0.0623 ±0.014	0.2317 ±0.095
Progesterone (4)	0.1250 ±0.049	0.1108 ±0.063	0.6305 ±0.142
Progesterone + Estradiol-17β (4)	0.1943 ±0.049	0.1433 ±0.087	0.5212 ±0.078

*Pico moles of steroid bound at saturation point/mg protein of tissue homogenate.

( ) = No. of animals.

**Column means are not significantly different ($P > 0.05$).

Table 8. Effect of progesterone and progesterone + estradiol-17β vaginal device treatment on the presence of estrogen and progesterone receptor sites* in the anterior pituitary of prepuberal heifers.

Treatment	Estrogen Receptors	Progesterone Receptors	
	^{3}H-E$_2$ Bound	^{3}H-P Bound	^{3}H-R5020 Bound
Control (3)	0.0857 ±0.037	0.0493 ±0.008	0.0770 ±0.008
Progesterone (4)	0.1655 ±0.041	0.0908 ±0.026	0.1495 ±0.036
Progesterone + Estradiol-17β (4)	0.1418 ±0.008	0.0910 ±0.075	0.2368 ±0.174

*Pico moles of steroid bound at saturation point/mg protein of tissue homogenate.

( ) = No. of animals.

**Column means are not significantly different ($P > 0.05$).

Table 9. Effect of progesterone and progesterone + estradiol-17β vaginal device treatment on the presence of estrogen receptor sites* in the anterior hypothalamus of prepuberal heifers.

Treatment	Estrogen Receptors ^{3}H-E$_2$ Bound
Control (3)	0.086 ±0.047
Progesterone (4)	0.180 ±0.083
Progesterone + estradiol-17β (4)	0.214 ±0.130

*Pico moles of steroid bound at saturation point/mg protein of tissue homogenate.

( ) = No. of animals.

**Means are not significantly different ($P > 0.05$).

DISCUSSION

Following insertion of silastic devices containing progesterone, the serum progesterone levels indicate that the release of progesterone from silicone rubber depends mainly on the surface area of the device. This is in agreement with Dziuk and Cook (1966) who also reported that surface area is more important than concentration in determining release of steroids from silicone rubber implants. Similarly, Roche (1974) reported that the silastic devices with large surface area prevented heifers from coming into estrus, whereas devices having half the surface area did not prevent heifers from coming into estrus. Studies on the transport of steroids across silicone membranes have also shown that permeability rates were inversely proportional to thickness of the membrane (Kincl and Rudel, 1971), influenced by the structure of the steroid (Sundaram and Kincl, 1968) and degree of lyophilicity of the steroid (Kincl and Rudel, 1971). The present study is the first report which shows that route of administration of the progesterone devices whether subcutaneous or intravaginal does not affect the release of progesterone through silicone membrane.

A decrease in peripheral level of serum progesterone over time was observed while the devices were in place. A similar observation was made in cycling heifers (Rajamahendran et

al., 1975), goats (Dhindsa et al., 1973), sheep (Symons et al., and prepuberal gilts (Beebe, 1976). Although Symons et al. (1974) reported that the decline in serum progesterone was due to a physiological process rather than to unavailability of the hormone from implants, the reason for the decline is not clear. However, several possibilities exist. Either the transport of progesterone from silastic device was affected due to (a) encapsulation by fibrous connective tissue and secretions (Kincl and Rudel, 1971), (b) absorption of lipid components from the tissue to the device (Kincl and Rudel, 1971), (c) the thickness of the silastic tubing or rapid removal of progesterone from circulation by increased liver enzyme activity and conjugation.

Diurnal variation in serum progesterone was demonstrated following insertion of progesterone devices. Diurnal variation in serum progesterone levels did not occur in the bovine at estrus (Katangolle et al., 1974) or during mid-luteal phase (Rajamahendran and Baker, 1975). The reason for the observed diurnal variation in the present study is not clear. The most likely possibility would be the differences in metabolic activity and liver conjugation of progesterone which is believed to vary with feeding pattern and animal behaviour (MacAdam and Eberhart, 1972) or differences in blood supply to various parts of the body at different times of the day. A diurnal variation in peripheral plasma concentration of adrenal corticoids has been

demonstrated in man (Rose et al., 1972), pigs (Bottoms et al., 1972) and horses (Bottoms et al., 1972). However, in the bovine results are conflicting. Hudson et al. (1974) failed to observe any diurnal variation. MacAdam and Eberhart (1972) obtained an early morning peak but no following trough, whereas Wagner and Oxenreider (1972) obtained a trough between 1800 hr and 0200 hr, but no early morning peak. It is also possible that the diurnal variation observed in the present study was due to progesterone produced by the adrenals. It has been shown that adrenals produce progesterone in bovine (Balfour and Comline, 1957). Resko (1969) reported that plasma progesterone level increased following ACTH in ovariectomized rats. Thus, one might postulate that heifers may also experience a diurnal progesterone level as a result of change in adrenal activity.

It has been suggested that serum progesterone concentration of 1 ng/ml and above will block estrus and ovulation in cycling bovine (Hansel and Malven, 1970). Further, the serum progesterone profile during the estrous cycle in the bovine (Stabenfeldt, 1969; Henrichs et al., 1971; Wettemann et al., 1971; Rajamahendran et al., 1976) also indicates that estrus and ovulation occur only after the serum progesterone declines to less than 1 ng/ml. In the present study except for treatment "A" prepuberal heifers, the serum progesterone in other heifers at device removal was greater than 1 ng/ml. Therefore, the

devices used in treatment "B", "C" and "D" may be used to control estrus and ovulation in cycling heifers. However, the practical difficulties involved in inserting the treatment "B" device do not favour its use in estrus synchronization.

None of the prepuberal heifers treated with saline following progesterone device treatment exhibited estrus or ovulated, suggesting that the progesterone priming should be followed with an ovulatory stimulus. This is in agreement with other workers who indicated that progesterone pretreatment increased the incidence of ovulation in prepuberal heifers (Howe et al., 1962; Arije et al., 1969; Neville and Williams, 1973) and anestrus ewes (Robinson, 1959; Gordon, 1963; Hulet and Foote, 1969). The number of ovulations following 500 IU of PMSG ranged from two to four and no unovulated follicles were observed at palpation, which suggests that progesterone treatment also suppressed excess follicular development. The results suggest that more uniform ovulations with excess follicular development can be obtained in progesterone primed prepuberal heifers with increased dose of PMSG. Gonzalez-Padilla (1975b) reported that all prepuberal heifers which received estradiol-17β with progesterone priming ovulated. In contrast, in the present study only 25% of the estradiol-17β treated heifers following progesterone priming ovulated. The low success rate obtained may be due to the differences in age of the heifers used.

Recent experience by the author indicates that estrus, ovulation and cyclic activity can be induced in prepuberal yearling heifers by treating them for 12 days with vaginal device containing estradiol-17β and progesterone. Kaltenbach et al. (19[redacted] have shown GnRH to produce substantial increase in serum FSH and LH levels in heifers following administration of GnRH. It was reported (Bedirian, 1973) that GnRH stimulated follicular growth but did not cause any ovulation in prepuberal heifers. However, in the present study 25% of the heifers treated with GnRH following progesterone priming ovulated. This observation is encouraging. Further work should be done with higher doses of GnRH in an attempt to induce ovulation in progesterone primed heifers.

Serum estradiol-17β observed in cycling heifers with vaginal device containing progesterone + estradiol-17β was thrice the previous reported value for proestrus heifers (Wettemann et al.,1972). Serum estradiol-17β declined to pretreatment levels forty-eight hours after insertion of the device and this value was comparable to basal values reported for normal cycling heifers (Wettemann, 1972). The maximum concentration of serum progesterone observed was comparable to mean progesterone concentration previously reported (Henricks et al., 1971; Wettemann et al., 1972; Rajamahendran et al., 1976) for heifers in mid-luteal phase of the cycle. Twenty-four hours after removal of the device progesterone concentration declined to

basal values reported for proestrus heifers (Henricks et al., 1971; Wettemann et al., 1972; Rajamahendran et al., 1976). The steady continuous release rate of progesterone from silastic vaginal device appears to simulate more closely the normal endogenous release rate of steroids from endocrine glands than daily injections.

The high percentage (> 90%) of heifers exhibiting estrus following a 12-day treatment with vaginal device containing progesterone + estradiol-17β observed in this study is comparable to others' results (Wiltbank and Kasson, 1968; Roche, 1974; Wiltbank and Gonzalez-Padilla, 1975). The percentage of heifers exhibiting estrus and ovulation and the interval to estrus after device removal was the same regardless of the stage of the cycle. However, Roche (1974), using subcutaneous implants containing progesterone, inserted for 12 days and an injection of estradiol benzoate at insertion, observed that the estrus response was low in animals treated on day 3 and 17 but was high in those treated during mid-luteal phase of the estrous cycle. He also reported that progesterone and estrogen injection at the time of insertion of subcutaneous implants containing progesterone increased the estrus response in heifers treated on day 17. Similarly, in the present study an injection of progesterone and estradiol benzoate given at the time of insertion of vaginal device containing progesterone was capable of synchronizing estrus in 90% of

the treated heifers.

Progesterone injection at the time of device insertion was given to shorten the length of cycle in heifers that were in day 0 to 4 of the cycle (Harms and Malven, 1969; Ray et al., 1961; Woody et al., 1967) and to suppress estrogen induced LH release in follicular phase heifers. High levels of progesterone apparently can inhibit the LH response to estradiol (Bolt et al., 1971; Scaramuzzi et al., 1971; Hobson and Hansel, 1971). The high estrus response observed in heifers treated with vaginal device containing progesterone + estradiol-17β during early luteal phase and follicular phase indicate that the progesterone released from the device was sufficient to shorten the length of the cycle and to suppress estrogen induced LH release. The results indicate that both the devices used in the present study were effective in synchronizing estrus in cycling heifers. However, vaginal device containing progesterone and estradiol-17β seems to be more practicable than injections of estradiol benzoate and progesterone given along with vaginal device containing progesterone.

Based on limited number of observations the conception rate following synchronized estrus was satisfactory. This conception rate is in agreement with conception rate for replacement heifers in our herd at first insemination. Conception rate comparable to controls were also observed by other workers

using 9-day (Wiltbank and Kasson, 1968) and 12-day (Roche, 1974) progestagen treatment. The conception rate to matings at the controlled estrus following 18 to 21 days progestagen treatment was lower than normal (Hansel, 1967; Jochle, 1972). Following short term progesterone treatment Rodetter et al. (1972) observed that plasma estrogen levels at estrus did not differ from control, whereas heifers treated with progestagen for 18 to 21 days had very high levels of estrogen. The abnormally high estrogen levels have been attributed to the low conception rate observed with long term progestagen treatment. The high estrogen levels have resulted in reduced sperm transport (Quinlivan and Robinson, 1969), fertilization rate (Hill et al., 1971) and altered transport of ova through the oviduct (Reed and Rich, 1972)

The high (90%) retention rate of vaginal device in heifers obtained in the present study was comparable to values reported for Abbott vaginal coils (Mauer et al., 1975; Rajamahendran et al., 1975; Roche, 1976). Although the retention rate in cows was not tested in this study, others (Sreenan, 1974; Roche, 1976) indicated that the retention rate in cows was slightly lower than heifers. The lower retention rate may be due to increased vaginal dimension, which seems to increase with calving. Further work should be done to evaluate the retention rate and estrus synchronization success in cows using vaginal device. Vaginal

devices used in the present study were more easily inserted and removed than the Abbott Vaginal coils. Furthermore, there was less irritation to the vaginal wall as judged by rectal palpation and no uneasiness observed in heifers while the devices were in place. The only adverse effect noted was occasional yellowish white discharge during the treatment period particularly when animals were housed.

It has been shown (Cooper and Furr, 1974; Lauderdale, 1974; Rajamahendran and Baker, 1975) that two injections of prostaglandin $F_{2\alpha}$ 10 to 12 days apart without reference to previous cycle resulted in the majority of animals returning to estrus 48 to 72 hours after second injection. The conception rate following synchronized estrus based on limited observations appear to be normal. However, the high cost of $PGF_{2\alpha}$ ($30 per animal) and the practical problem of giving injections will not favour its use in large scale beef operations. Further, it does not appear that Food and Drug Administration will allow the use of prostaglandin $F_{2\alpha}$ for estrous synchronization in cattle, for its known side effects and misuse in humans. The vaginal device used in the present study is easy to make, easily inserted and removed and costs only five dollars per device and it contains progesterone and estradiol-17β, which are normally present in blood, milk and tissue.

From extensive studies in the ovariectomized ewe (Robinson,

1959) it is now clear that both progesterone and estradiol-17β play an important role in the induction of estrous behaviour. In the present study all ovariectomized heifers treated with vaginal device containing estradiol-17β exhibited estrus approximately 24 hours after the insertion of the devices, whereas none of the heifers treated with vaginal devices containing progesterone or progesterone + estradiol-17β exhibited signs of estrus after insertion. The results suggest that progesterone could block estrogen induced estrous behaviour. This observation agrees with other reports in ovariectomized heifers (Short et al., 1973) and ovariectomized ewes (Scaramuzzi et al., 1971). The site of action of progesterone block of estrous behaviour is not known. It has been reported (Astwood and Dempsey, 1941; Dey et al., 1942) that estrous behaviour can be induced in estrogen treated ovariectomized females regardless of the presence of a functional pituitary gland. Hence progesterone block of estrus must be at the hypothalamic level.

In the present study higher serum estradiol-17β was observed in ovariectomized heifers which received estradiol-17β device relative to that in heifers receiving estradiol-17β with progesterone. The exact reason for the difference is not clear but it may be possible that progesterone either affected release of estradiol-17β from the vaginal device or the absorption of estradiol-17β through the vaginal wall. Vaginal device

containing estradiol-17β alone induced a significant release of serum LH 16 to 20 hours after insertion of the device. This observation is in agreement with other reports in ovariectomized heifers (Short et al., 1973; Hausler and Malven, 1976) and prepuberal heifers (Swanson, 1974; Gonzalez-Padilla et al., 1975). These observations and the blood hormone levels strongly suggest that estrogen causes LH release in the bovine. The observation that progesterone device treatment alone did not affect the concentration of serum LH is in agreement with others for ovariectomized cows (Hobson and Hansel, 1972; Short et al., 1973; Hausler and Malven, 1976) and cycling cows (Hobson and Hansel, 1971).

The pituitary LH response of cycling heifers to a relatively low dose of estrogen was lowered during the luteal phase of the estrous cycle (Hobson and Hansel, 1972). Similarly, in the present study progesterone was able to block estrogen induced LH release in all ovariectomized heifers. This study confirms the findings of others in ovariectomized ewe (Scaramuzzi et al., 1971; Diekman and Malven, 1973) and intact ewe (Bolt et al., 1971; Howland et al., 1971). However, with similar experimental model Short et al. (1973) and Hausler and Malven (1976) failed to inhibit estrogen induced LH release by exogenous progesterone in ovariectomized heifers. The present study clearly demonstrates that, in the bovine, progesterone blocks estrogen induced LH

release at anterior pituitary or hypothalamic level.

The serum estradiol, progesterone and LH observed in the present study in control prepuberal heifers were comparable to others' findings (Donaldson et al., 1970; Swanson et al., 1972; Gonzalez-Padilla et al., 1975). It has been reported (Gonzalez-Padilla et al., 1975) that estradiol administration results in estrous behaviour and LH peak in prepuberal heifers without subsequent ovulation. The failure to observe estrus and LH peak in prepuberal heifers treated with estradiol-17β progesterone vaginal device suggests that progesterone blocked estradiol induced estrus and LH release. Two prepuberal heifers that were treated with progesterone + estradiol-17β device for 12 days showed LH peak after removal of the device. Gonzalez-Padilla et al. (1975) observed, in prepuberal heifers near puberty, peaks of serum progesterone and LH peaks when the serum progesterone declined.

Target tissues for steroids contain specific receptor molecules which have a higher affinity for steroid molecule. In rats specific receptors for estrogens and progesterone are found in the reproductive tract, the mammary gland, the anterior pituitary and hypothalamus (O'Malley, 1971). These receptors are present in relatively low numbers within the target tissue cells. Binding curves for uterine, anterior pituitary and hypothalamic cytosol preparations in bovine indicate that the

progesterone and estrogen binding proteins from the above tissues are saturable at concentration of progesterone and estrogen within the physiological range. Steroid specificity of the receptor was demonstrated by employing 100-fold excess of cold steroid. The tissue specificity of the steroid was demonstrated by showing high receptor content in uterus, low in anterior pituitary and hypothalamus and non-detectable levels in liver and cerebral cortex. These criteria were used to validate receptor assay in this study.

Estrogen binding protein concentration observed in uterine cytosols fall within the range of concentrations reported for calf uterus (Sanborn et al., 1971) and endometrium of cycling heifers (Kimball and Hansel, 1974). In the present study a significant decrease in uterine estrogen binding protein was observed in progesterone and progesterone + estradiol-17β treated heifers compared to controls. This observation is in agreement with others, who reported that progesterone treatment caused a significant reduction in the amount of cytoplasmic estrogen receptors in immature rat uterus (Huseh et al., 1976), rabbit uterus (Martin, et al., 1975). In the present study the progesterone binding concentration observed for uterine cytosols is similar to that reported for calf uterus (Hays, 1975). In this study a significant decrease in uterine progesterone binding protein was observed in progesterone and progesterone +

estradiol-17β treated heifers compared to control. This observation is in agreement with other reports in rats (Parks et al., 1974), guinea pig (Milgrom et al., 1972), hamster (Kato, 1975).

The present study is the first report of anterior pituitary and hypothalamus cytosol estrogen and progesterone binding proteins in the bovine. Therefore, it is not possible to draw comparisons except with reports of similar binding protein in sheep and goats (Galscock and Hoekstra, 1969) and rats (Kato and Villee, 1967) and other species. The demonstration of specific estrogen and progesterone binding protein in the anterior pituitary and hypothalamus suggests that progesterone and estrogen modulate the secretion of gonadotrophins by acting at these areas. In this study no significant change in the estrogen and progesterone binding protein were observed in anterior pituitary and hypothalamic cytosols in progesterone and progesterone + estradiol-17β treated heifers. However, in the anterior pituitary and anterior hypothalamic cytosols, a slight increase in estrogen receptor protein was observed in progesterone and progesterone + estradiol-17β treated heifers. Similar observations have not been reported in other species. However, it has been shown that estrogen pretreatment enhances tissue sensitivity to progesterone and this enhancement is correlated with an increased quantity of cytoplasmic receptor for progesterone (Milgrom et al., 1973).

In the present study it was hypothesized that progesterone pretreatment would increase the receptors for estrogen at the anterior pituitary and/or hypothalamic level. Although there is a trend towards increased estrogen receptor concentration the results obtained were not significant, probably due to small number of observations made. Further work should be done with greater numbers of animals and employing increased concentration of anterior pituitary and hypothalamic cytosols. However, the effect of progesterone priming can also be explained by a mechanism other than changes at the hypothalamic or anterior pituitary level, since regardless of progesterone priming, the estradiol injection elicited LH peaks in prepuberal heifers (Swanson et al., 1974; Gonzalez-Padilla et al., 1975). Therefore, the possibility exists that progesterone priming may increase the sensitivity of the ovaries to endogenous gonadotrophin thereby increasing follicular development and ovulation or both mechanisms may exist.

It has been demonstrated that 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione (R 5020) binds to the rabbit and guinea pig uterus cytosol progesterone receptor with an intrinsic association constant two to five times higher than that of progesterone (unpublished data). The present study confirms the above reports in bovine uterus, anterior pituitary and hypothalamus cytosols.

In conclusion, a relatively simple and inexpensive vaginal device containing progesterone + estradiol-17β was developed to synchronize estrus in cattle. There was precise onset of estrus after removal of the device and hence will render artificial insemination feasible at predetermined time without prior detection of estrus. The fertility rate following vaginal device, based on small number of observations is satisfactory. Further work should be done to determine the retention rate, effective synchronization and conception rate under field conditions. Preliminary studies indicate that this vaginal device could be used to induce fertile estrus in postpartum anestrus cows. The devices were not tested for retention rate in cows. If retention rate of the device becomes a problem, it might be improved by increasing the surface area of the device and/or thickness of the silastic tubing. Studies also indicate that these devices could be used to induce cyclic activity in prepuberal and anestrus heifers. Furthermore the devices can be used to study the role of ovarian steroids in induction of puberty, parturition, lactation and other physiological function.

The present study has demonstrated that progesterone block estrogen induced estrus behaviour and LH release. However, the role of progesterone in the initiation of puberty is yet to be determined. It is suggested that in view of the small number

of animals used and the observed high variability, further work be done using more animals. However, the possibility exists that progesterone may be involved in the final events leading to puberty by increasing the sensitivity of the ovaries to endogenous gonadotrophins, thereby increasing follicular development or both mechanisms may exist.

CLAIM OF ORIGINALITY

To the best of the author's knowledge, the following elements, in the thesis, constitute original contributions to knowledge.

1. Estrus was synchronized in cycling heifers using a silastic vaginal device containing progesterone and estradiol-17β.
2. The demonstration that progesterone blocks estrogen induced estrus behaviour and LH surge in ovariectomized and prepuberal heifers.
3. The observation that route of insertion of silastic device containing progesterone does not influence serum progesterone.
4. Induction of ovulation and cyclic activity in progesterone primed, prepuberal heifers using a synthetic gonadotrophin releasing hormone (GnRH).
5. The demonstration of diurnal variation in serum progesterone in prepuberal heifers following silastic device containing progesterone.
6. The demonstration of a decrease in uterine estrogen cytosol binding protein following progesterone treatment in prepuberal heifers.
7. The demonstration of specific progesterone and estrogen cytosol binding protein in anterior pituitary and hypothalamus of prepuberal heifers.

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APPENDIX TABLES

Appendix Table 1A. Expt. I. Serum progesterone before, during and after single subcutaneous device (Treat. "A").

Day and Time of Sample		Animal Number							
		72	76	151	153	156	158	51	54
D-1	6 AM	0.4	0.8	0.7	1.2	0.8	0.5	1.0	0.8
	12 N	0.4	0.4	0.5	0.6	0.6	0.4	0.6	0.6
	6 PM	0.5	0.5	0.6	0.5	0.7	0.8	0.5	0.5
	12 MN	0.6	0.6	0.4	0.7	0.9	0.9	0.7	0.5
D_1		1.0	2.4	2.0	1.8	0.8	1.6	2.1	1.8
D_2		1.2	2.2	1.6	2.0	1.1	1.6	2.3	0.9
D_4	6 AM	0.9	0.8	1.9	1.2	0.9	1.2	2.0	1.5
	12 N	0.7	0.5	1.7	1.4	1.7	1.3	1.4	1.2
	6 PM	0.7	0.5	1.3	1.3	1.1	1.3	0.9	1.0
	12 MN	0.6	0.8	0.8	0.9	0.6	0.8	0.8	1.5
D_8	6 AM	0.7	0.6	1.5	0.8	1.0	0.7	1.5	0.9
	12 N	0.4	0.5	0.9	0.7	0.9	0.8	1.0	0.7
	6 PM	0.5	0.4	1.2	0.6	1.0	0.9	1.4	0.8
	12 MN	0.5	0.3	1.4	1.4	0.8	1.0	0.9	0.7
D_{12}	6 AM	0.7	0.6	0.8	1.1	1.2	0.8	1.4	1.0
	12 N	0.8	0.4	1.0	0.9	0.8	0.7	1.2	0.8
	6 PM	0.5	0.3	0.6	0.5	0.7	0.6	1.1	1.5
	12 MN	0.6	0.4	0.9	0.9	1.4	1.2	1.3	1.1
D_{16}	6 AM	0.5	0.4	0.8	1.1	1.1	0.9	0.9	0.8
	12 N	0.6	0.3	0.6	0.6	0.6	0.5	0.8	0.7
	6 PM	0.4	0.4	0.6	0.6	0.6	0.6	0.9	0.9
	12 MN	0.3	0.5	0.4	0.5	0.5	0.7	1.0	1.0
D_{20}	6 AM	0.6	0.5	0.8	0.7	0.9	0.5	0.7	0.8
	12 N	0.5	0.4	0.6	0.5	0.6	0.6	0.6	0.9
	6 PM	0.5	0.6	0.6	0.6	0.8	0.7	0.9	0.9
	12 MN	0.4	0.4	0.5	0.4	0.6	0.8	0.8	0.5
D_{21}	6 AM	0.5	0.5	0.7	0.6	0.6	0.4	0.6	0.7
	12 N	0.4	0.4	0.4	0.3	0.4	0.6	0.8	0.6
	12 MN	0.5	0.3	0.5	0.6	0.5	0.3	0.5	0.7

continued

Appendix Table 1B. Expt. I. Serum progesterone before, during and after double subcutaneous device (Treat. "B").

Day and Time of Sample		Animal Number							
		69	73	48	53	154	157	168	160
D-1	6 AM	0.7	0.6	0.4	0.4	0.8	0.6	0.5	0.4
	12 N	0.6	0.7	0.5	0.3	0.7	0.7	0.8	0.6
	6 PM	0.6	0.4	0.3	0.4	0.4	0.6	0.4	0.4
	12 MN	0.5	0.5	0.4	0.5	0.6	0.4	0.6	0.3
D_1		3.0	2.4	2.6	2.2	3.4	3.1	3.4	4.0
D_2		2.8	2.5	2.6	2.0	3.1	2.8	2.9	3.0
D_4	6 AM	1.6	1.2	3.2	3.7	3.3	3.5	2.8	3.6
	12 N	1.9	1.1	1.7	1.8	3.5	3.3	3.2	3.2
	6 PM	1.8	1.3	1.8	1.9	2.6	3.4	3.0	3.0
	12 MN	1.7	1.5	2.3	1.7	2.4	2.5	2.4	2.8
D_8	6 AM	2.2	1.1	1.4	2.8	2.6	1.6	1.9	2.4
	12 N	1.2	1.2	2.2	1.9	1.7	1.8	2.3	2.3
	6 PM	1.4	1.3	1.8	1.8	1.8	3.0	2.0	1.8
	12 MN	1.6	2.1	1.5	1.9	1.3	1.4	1.6	1.2
D_{12}	6 AM	2.1	1.9	3.2	3.3	3.0	2.1	2.4	3.1
	12 N	0.8	1.4	2.1	2.9	2.7	2.2	2.0	3.3
	6 PM	1.6	1.6	1.8	2.5	1.7	1.8	1.6	2.4
	12 MN	1.9	1.8	2.0	3.2	1.9	2.4	1.4	2.0
D_{16}	6 AM	3.1	2.9	2.4	1.8	1.9	3.0	2.4	1.8
	12 N	1.2	1.3	2.1	1.6	1.5	2.7	1.8	1.7
	6 PM	1.2	1.5	1.4	1.4	1.9	2.6	1.9	1.4
	12 MN	1.4	1.2	1.2	2.4	1.5	2.2	2.2	1.8
D_{20}	6 AM	2.7	3.1	2.0	1.8	1.7	1.1	1.6	1.6
	12 N	2.5	1.7	2.0	1.6	1.5	0.8	1.0	1.3
	6 PM	1.7	1.6	0.9	1.3	1.2	1.6	0.9	1.9
	12 MN	1.2	1.5	0.8	0.9	2.2	0.8	1.1	1.0
D_{21}	6 AM	0.7	0.6	0.7	0.8	0.9	0.8	0.9	0.7
	12 N	0.6	0.5	0.6	0.7	0.7	0.7	0.6	0.5
	12 MN	0.5	0.5	0.3	0.6	0.5	0.4	0.3	0.5

continued

Appendix Table 1c. Expt. I. Serum progesterone before, during and after silastic vaginal device (Treat. "C").

Day and Time of Sample		Animal Number							
		93	101	52	55	161	163	166	169
D-1	6 AM	0.8	0.6	0.8	1.3	0.7	0.5	0.5	0.6
	12 N	0.8	0.7	0.7	1.2	0.6	0.5	0.6	0.8
	6 PM	0.8	0.5	0.6	1.4	0.5	0.4	0.5	0.7
	12 MN	0.6	0.8	0.5	0.8	0.6	0.8	0.5	0.9
D_1		3.1	4.2	3.6	3.2	3.4	2.8	3.0	2.6
D_2		3.6	4.7	3.8	2.9	2.6	3.0	2.9	2.6
D_4	6 AM	3.2	2.2	3.8	3.6	3.7	4.1	3.0	2.7
	12 N	2.5	1.8	3.4	3.8	2.8	2.7	2.5	2.4
	6 PM	3.0	2.4	3.3	2.1	2.7	3.9	2.4	2.8
	12 MN	3.4	2.5	4.0	3.5	2.6	3.0	3.6	3.1
D_8	6 AM	1.5	1.5	3.4	3.7	3.2	4.0	1.8	2.4
	12 N	2.1	2.1	1.8	1.7	1.8	1.9	1.5	2.5
	6 PM	2.0	1.2	2.2	1.8	1.9	2.2	2.9	1.9
	12 MN	2.4	2.0	2.9	1.8	1.1	1.2	2.8	2.1
D_{12}	6 AM	2.0	1.1	1.9	1.5	2.0	2.7	3.0	2.7
	12 N	1.8	1.2	2.4	1.5	1.7	1.9	1.9	1.9
	6 PM	1.5	1.9	1.7	1.3	2.0	1.7	1.7	2.0
	12 MN	2.1	1.7	3.0	1.9	2.2	1.7	1.9	1.7
D_{16}	6 AM	2.8	0.9	1.1	1.2	1.2	1.2	1.2	2.0
	12 N	1.7	1.5	1.0	1.9	1.8	1.1	1.3	1.7
	6 PM	1.9	1.4	1.4	1.7	1.5	1.4	1.1	2.1
	12 MN	1.6	1.2	2.5	2.8	1.9	1.3	2.3	1.9
D_{20}	6 AM	2.4	1.8	1.1	1.5	1.3	1.5	0.9	2.0
	12 N	1.8	1.6	1.2	1.2	1.4	1.2	1.3	1.1
	6 PM	1.4	1.1	1.1	1.8	1.1	1.3	1.1	1.1
	12 MN	1.5	1.7	1.3	1.1	1.5	1.1	0.8	1.0
D_{21}	6 AM	0.8	0.9	0.8	0.6	0.6	0.8	0.6	0.9
	12 N	0.7	0.8	0.5	0.5	0.5	0.6	0.5	0.5
	12 MN	0.5	0.5	0.4	0.4	0.5	0.5	0.4	0.5

continued

Appendix Table 1D. Expt. I. Serum progesterone before, during and after Abbott vaginal device (Treat. "D").

Day and Time of Sample		Animal Number							
		98	99	61	56	162	165	170	160
D-1	6 AM	0.5	0.8	0.5	0.6	0.5	0.7	0.5	1.1
	12 N	0.9	0.9	0.7	0.5	0.4	0.7	0.7	0.8
	6 PM	0.9	0.9	0.7	0.6	0.4	0.4	0.7	0.7
	12 MN	0.8	0.7	0.4	0.7	0.3	0.7	0.5	0.9
D_1		6.2	5.6	5.1	5.8	5.0	4.6	6.3	6.1
D_2		5.9	5.8	5.7	5.9	5.0	5.2	6.0	6.2
D_4	6 AM	6.1	5.2	5.5	6.0	6.9	6.0	5.2	6.3
	12 N	5.2	4.9	3.9	5.2	6.3	4.7	6.4	5.7
	6 PM	5.2	3.7	3.7	4.1	5.9	4.3	6.1	4.2
	12 MN	5.4	3.6	3.2	5.8	2.9	5.2	5.9	5.3
D_8	6 AM	3.2	2.6	6.1	4.8	4.6	5.2	3.9	5.2
	12 N	2.3	3.5	4.2	3.8	6.5	4.7	4.4	4.8
	6 PM	4.1	4.2	4.1	4.3	4.9	4.8	3.4	4.9
	12 MN	4.2	4.5	5.8	3.3	3.2	5.1	4.9	4.5
D_{12}	6 AM	6.0	6.2	6.0	3.5	3.9	2.4	1.8	4.6
	12 N	6.2	6.1	6.1	4.6	3.7	2.7	1.7	3.8
	6 PM	4.5	2.9	4.3	3.6	3.5	1.8	1.2	4.2
	12 MN	6.1	5.5	3.4	5.2	3.9	2.5	2.4	2.9
D_{16}	6 AM	4.0	4.2	5.2	4.7	2.8	1.8	1.9	3.3
	12 N	1.9	1.5	3.3	4.0	1.8	2.2	2.1	3.2
	6 PM	1.8	1.5	3.2	2.7	2.5	2.0	1.7	2.9
	12 MN	3.8	3.1	5.1	4.0	2.1	3.0	2.1	3.7
D_{20}	6 AM	3.4	3.9	3.2	4.2	1.6	1.5	1.6	3.1
	12 N	1.7	2.2	3.0	2.7	2.9	1.6	2.1	2.4
	6 PM	1.4	1.2	2.1	3.7	1.8	1.1	2.7	2.1
	12 MN	2.4	2.7	3.1	3.9	1.6	1.5	1.2	1.0
D_{21}	6 AM	1.1	0.9	0.8	1.1	1.1	1.3	1.2	1.2
	12 N	0.8	0.6	0.6	0.8	1.0	0.8	0.8	0.7
	12 MN	0.5	0.3	0.4	0.5	0.6	0.6	0.7	0.5

Appendix Table 2A. Expt. I. Induction of estrus, ovulation and cyclic activity in prepuberal heifers post-progesterone priming.

Treatment	No. Treated	No. Exhibited Estrus	No. Ovulated	No. Returned to Estrus After 20-22 Days
Effect of Saline				
Single s/c device "A"	2	0	0	0
Double s/c device "B"	2	0	0	0
Vaginal device "C"	2	0	0	0
Abbott's vaginal device "D"	2	0	0	0
Effect of Estradiol-17β 5 mg I/M				
Single s/c device "A"	2	2	0	0
Double s/c device "B"	2	1	1	0
Vaginal device "C"	2	1	0	0
Abbott's vaginal device "D"	2	1	1	1
Effect of PMSG 500 IU I/M				
Single s/c device "A"	2	0	0	0
Double s/c device "B"	2	2	2	0
Vaginal device "C"	2	2	2	1
Abbott's vaginal device "D"	2	2	2	0
Effect of GnRH 100 µg I/M				
Single s/c device "A"	2	0	0	0
Double s/c device "B"	2	1	1	1
Vaginal device "C"	2	0	0	0
Abbott's vaginal device "D"	2	1	1	0

continued

Appendix Table 2B. Expt. I. Serum progesterone following induced estrus and ovulation

Animal No.	P. Device Treatment	Hormone Treat.	No. of Ovulation	Serum Progesterone ng/ml D_5	D_{10}	D_{15}	D_{22}
157	Treat "B"	Estradiol	1	2.0	3.2	3.6	2.8
55	Treat "D"	"	1	1.6	4.1	4.4	0.7
154	Treat "B"	GnRH	1	1.8	4.0	3.9	0.8
162	Treat "D"	"	1	2.1	3.6	3.8	3.2
73	Treat "B"	PMSG	1	2.0	4.8	5.2	5.4
168	Treat "B"	"	3	3.6	9.0	6.8	6.2
56	Treat "D"	"	2	1.8	3.6	3.8	3.7
152	Treat "D"	"	3	1.9	5.2	5.1	3.8
166	Treat "C"	"	2	2.1	5.1	4.2	0.6
52	Treat "C"	"	4	5.6	5.9	7.3	7.8

Appendix Table 3A. Expt. I. Progesterone conc. following silastic progesterone devices. Analysis of variance table.

Tests	Source	DF	Sum of Squares	Mean Square	F Value	Prob F
Numerator	Season	1	0.03255	0.032552	0.01557	0.9003
Denominator	Error A	6	12.54224	2.090373		
Numerator	Day	5	402.73776	80.547552	281.93117	0.0001
Numerator	Time	3	18.36260	6.120868	21.42416	0.0001
Numerator	Day/time	15	8.16693	0.544462	1.90572	0.0271
Numerator	Season/day	5	17.39448	3.478896	12.17677	0.0001
Numerator	Season/time	3	3.97922	1.326406	4.64266	0.0043
Numerator	Season/day/time	15	5.36687	0.357792	1.25234	0.2406
Denominator	Error B	138	39.42651	0.285699		
Numerator	Treat	3	583.05104	194.350347	722.53397	0.0001
Numerator	Day/treat	15	186.11849	12.407899	46.12870	0.0001
Numerator	Time/treat	9	7.47469	0.830521	3.08762	0.0016
Numerator	Day/time/treat	45	13.08516	0.290781	1.08103	0.3389
Numerator	Season/treat	3	14.60141	4.867135	18.09449	0.0001
Numerator	Season/day/treat	15	38.24844	2.549896	9.47972	0.0001
Numerator	Season/time/treat	9	4.80432	0.533814	1.98455	0.0592
Numerator	Season/day/time/treat	45	12.28521	0.273005	1.01495	0.4497
Denominator	Error C	432	116.20125	0.268984		

continued

Appendix Table 3B. Expt. I. Progesterone conc. following silastic progesterone implants. Overall means.

Season	Day	Time	No.	Progesterone ng/ml
	0	6	32	0.67500000
	0	12	32	0.64062500
	0	18	32	0.58750000
	0	24	32	0.60625000
	4	6	32	3.33750000
	4	12	32	2.93125000
	4	18	32	2.70937500
	4	24	32	2.72187500
	8	6	32	2.52500000
	8	12	32	2.19062500
	8	18	32	2.24062500
	8	24	32	2.23125000
	12	6	32	2.50000000
	12	12	32	2.28750000
	12	18	32	1.89375000
	12	24	32	2.26562500
	16	6	32	2.04062500
	16	12	32	1.58125000
	16	18	32	1.53437500
	16	24	32	1.91250000
	20	6	32	1.75312500
	20	12	32	1.45312500
	20	18	32	1.33750000
	20	24	32	1.31875000

continued

Appendix Table 3B.

Season	Day	Time	No.	Progesterone ng/ml
S		6	96	2.07083333
S		12	96	1.89375000
S		18	96	1.79895833
S		24	96	1.75625000
W		6	96	2.20625000
W		12	96	1.80104167
W		18	96	1.63541667
W		24	96	1.92916667
		6	192	2.13854167
		12	192	1.84739583
		18	192	1.71718750
		24	192	1.84270833

Appendix Table 3C. Expt. I. Analysis of variance for variable Hematocrit values.

Tests	Source		Sum of Squares	Mean Square	F Value	Prob F
Numerator	Day	5	49.36927	9.873854	2.00970	0.0867
Numerator	Time	3	25.22458	8.408194	1.71139	0.1709
Numerator	Day/time	15	60.85198	4.056799	0.82571	0.6471
Denominator	Error A	72	353.74250	4.913090		
Numerator	Treat	3	552.76146	184.253819	34.44511	0.0001
Numerator	Day/treat	15	103.86885	6.924590	1.29451	0.2068
Numerator	Time/treat	9	11.08812	1.232014	0.23032	0.9890
Numerator	Day/time/treat	45	38.44406	0.854313	0.15971	1.0000
Denominator	Error	216	1155.42750	5.349201		

Appendix Table 4. Expt. II. Serum progesterone and estradiol-17β in cycling heifers treated with intravaginal device containing progesterone + estradiol-17β.

Blood Sampling Schedule	Serum Progesterone ng/ml					Serum Estradiol pg/ml				
	270*	273	274	275	Mean ± S.D.	270	273	274	275	Mean ± S.D.
-24 hr	1.2	3.4	1.7	2.4	2.2 ± 0.9	6.2	6.7	6.1	3.7	5.7 ± 1.3
0 hr**	1.8	4.4	2.2	2.2	2.7 ± 1.2	5.6	6.7	5.3	6.1	5.9 ± 0.6
12 hr	5.9	6.0	4.9	6.6	5.9 ± 0.7	36.9	28.2	13.2	39.8	29.5 ± 11.9
24 hr	5.8	6.6	4.8	5.2	5.6 ± 0.8	26.8	8.9	25.5	47.4	27.2 ± 15.7
Day 2	3.0	2.0	2.8	3.2	2.8 ± 0.5	6.9	4.8	10.8	7.1	7.4 ± 2.5
Day 4	2.6	2.0	2.9	2.0	2.4 ± 0.4	6.1	6.6	8.6	6.2	6.9 ± 1.2
Day 8	2.1	1.6	2.0	2.3	2.0 ± 0.3	4.7	7.0	4.3	11.3	6.8 ± 3.2
Day 12***	1.1	1.8	1.4	1.0	1.3 ± 0.4	12.8	5.9	8.8	8.2	8.9 ± 2.9
Day 13	0.5	0.6	0.7	0.6	0.6 ± 0.1	8.1	15.0	7.7	8.6	9.9 ± 3.5

*Animal No.

**Time of insertion of device.

***Time of removal of device.

Appendix Table 5A. Expt. III. Serum estradiol-17β in ovariectomized heifers post-progesterone intravaginal device treatment.

Blood Sampling Schedule	Serum Estradiol-17β in pg/ml						
	148*	21	136	7	22	28	Mean±S.D.
-24 hr	5.1	5.5	3.9	3.8	8.0	4.1	5.1 ± 1.6
0 hr**	4.9	4.1	4.8	8.2	7.5	3.4	5.5 ± 1.9
4 hr	7.0	1.4	3.5	4.0	9.0	3.1	4.7 ± 2.7
8 hr	1.6	2.1	5.9	8.2	6.1	8.0	5.3 ± 2.8
12 hr	5.1	7.0	5.6	2.1	4.1	2.6	4.4 ± 1.9
16 hr	4.8	3.1	7.2	3.1	6.1	7.2	5.3 ± 1.9
20 hr	7.3	2.9	4.3	2.9	8.0	3.1	4.8 ± 2.3
24 hr	5.8	4.0	3.6	6.5	3.9	4.2	4.7 ± 1.2
28 hr	3.8	3.5	3.2	6.1	7.2	5.6	4.9 ± 1.6
32 hr	3.1	8.8	6.2	3.8	4.6	6.7	5.5 ± 2.1
36 hr	9.1	2.6	7.9	2.1	8.0	2.1	5.3 ± 3.3
48 hr	3.3	6.9	5.2	6.1	4.5	4.8	5.1 ± 1.3
Day 4	3.1	6.4	7.2	3.2	4.8	2.1	4.5 ± 2.0
Day 8	4.7	5.6	3.9	9.1	4.2	2.8	5.1 ± 2.2
Day 12*** 0 hr	3.3	4.3	7.8	8.1	7.5	3.7	5.8 ± 2.2
Day 13 0 hr	6.1	1.7	7.0	3.8	3.9	8.1	5.1 ± 2.4

*Animal No.

continued

**Time of insertion of device.

***Time of removal of device.

Appendix Table 5B. Expt. III. Serum estradiol-17β in ovariectomized heifers post-estradiol-17β intravaginal device treatment.

Blood Sampling Schedule	Serum Estradiol-17β in pg/ml						
	136*	22	148	28	21	7	Mean±S.D.
-24 hr	3.6	5.3	6.1	9.6	6.9	8.3	6.6 ± 2.1
0 hr**	7.8	7.9	6.4	9.1	6.2	8.2	7.6 ± 1.1
4 hr	41.9	44.8	35.5	30.8	55.7	37.1	40.9 ± 8.7
8 hr	13.4	21.4	50.8	30.9	32.4	24.9	28.9 ±12.7
12 hr	11.5	16.3	23.1	12.3	19.8	28.7	18.6 ± 6.6
16 hr	11.6	6.7	14.2	13.9	14.8	12.5	12.3 ± 3.0
20 hr	14.5	12.4	11.0	13.9	20.9	14.8	14.6 ± 3.4
24 hr	17.2	12.2	9.1	10.8	26.1	12.8	14.7 ± 6.2
28 hr	6.6	9.9	10.6	11.2	19.2	24.7	13.7 ± 6.8
32 hr	9.3	7.1	11.8	9.7	23.1	12.2	12.2 ± 5.7
36 hr	5.7	9.6	8.9	7.7	15.7	9.6	9.5 ± 3.4
48 hr	9.1	8.7	6.0	10.1	13.9	11.9	9.9 ± 2.7
Day 4	7.2	7.9	10.4	9.9	15.0	14.2	10.7 ± 3.2
Day 8	4.6	7.0	8.1	12.8	22.1	15.1	11.6 ± 6.3
Day 12*** 0 hr	5.5	7.4	8.9	7.3	10.9	6.8	7.8 ± 1.9
Day 13 0 hr	7.6	6.3	7.2	8.0	7.6	6.0	7.1 ± 0.8

*Animal No.

**Time of insertion of device.

***Time of removal of device.

continued

Appendix Table 5C. Expt. III. Serum estradiol-17β in ovariectomized heifers treated with intravaginal device containing progesterone + estradiol-17β.

Blood Sampling Schedule	Serum estradiol-17β in pg/ml						
	28*	7	22	144	136	148	Mean±S.D.
-24 hr	9.3	3.1	3.9	7.4	3.0	6.1	5.5 ± 2.5
0 hr**	3.2	6.2	3.3	6.7	3.5	5.4	4.7 ± 1.6
4 hr	24.4	30.8	25.5	52.9	25.7	33.1	32.1 ±10.7
8 hr	20.5	23.4	15.8	37.1	18.7	27.9	23.9 ± 7.7
12 hr	15.9	18.9	10.1	10.4	9.9	24.3	14.9 ± 5.9
16 hr	19.3	7.7	4.6	12.4	4.7	13.1	10.3 ± 5.7
20 hr	4.9	5.3	7.2	12.9	5.6	18.2	7.4 ± 3.0
24 hr	8.6	9.7	2.9	8.3	1.9	8.4	6.6 ± 3.3
28 hr	6.1	10.7	3.6	9.5	4.4	8.4	7.1 ± 2.9
32 hr	5.9	5.7	4.9	11.3	4.2	10.5	7.1 ± 3.0
36 hr	6.4	4.7	4.3	4.7	5.6	7.6	5.6 ± 1.3
48 hr	5.6	10.1	5.9	7.3	4.6	8.6	7.0 ± 2.1
Day 4	3.1	3.7	3.2	9.4	3.6	8.3	5.2 ± 2.8
Day 8	3.4	9.7	13.7	7.2	5.7	5.6	7.6 ± 3.6
Day 12*** 0 hr	3.1	5.7	4.2	8.5	2.7	6.2	5.1 ± 2.2
Day 13 0 hr	6.1	1.1	3.6	4.8	1.8	4.1	3.6 ± 1.8

*Animal No.

**Time of insertion of device.

***Time of removal of device.

Appendix Table 6A. Expt. III. Serum progesterone in ovariectomized heifers treated with intravaginal device containing progesterone.

Blood Sampling Schedule	Serum Progesterone Concentration in ng/ml						
	148*	21	136	7	22	28	Mean±S.D.
-24 hr	0.6	0.7	0.3	0.4	0.4	0.5	0.5 ± 0.1
0 hr**	0.6	0.9	0.3	0.3	0.4	0.5	0.5 ± 0.2
4 hr	4.8	5.2	2.8	2.2	5.2	3.2	3.9 ± 1.3
8 hr	4.8	4.4	4.8	6.2	4.6	3.4	4.7 ± 0.9
12 hr	5.8	5.2	1.6	3.0	4.2	3.4	3.9 ± 1.5
16 hr	3.6	3.0	2.4	2.6	3.8	3.4	3.1 ± 0.6
20 hr	3.0	2.8	2.2	4.6	5.0	3.8	3.6 ± 1.1
24 hr	4.2	2.6	4.2	2.0	3.8	2.4	3.2 ± 0.9
28 hr	3.0	3.2	2.8	1.6	3.2	2.8	2.8 ± 0.6
32 hr	3.8	3.8	2.0	2.2	4.4	3.1	3.2 ± 0.9
36 hr	4.2	2.8	1.4	1.2	3.2	2.2	2.5 ± 1.1
48 hr	1.6	2.4	2.4	2.0	3.2	4.0	2.6 ± 0.9
Day 4	1.6	2.1	2.1	2.0	2.1	2.8	2.1 ± 0.4
Day 8	1.8	2.2	1.2	3.5	1.6	2.4	2.1 ± 0.8
Day 12*** 0 hr	1.6	1.8	1.2	0.8	1.4	1.2	1.3 ± 0.3
Day 13 0 hr	0.6	0.5	0.4	0.3	0.8	0.7	0.6 ± 0.2

*Animal No.

continued

**Time of insertion of device.

***Time of removal of device.

Appendix Table 6B. Expt. III. Serum progesterone in ovariectomized heifers treated with intravaginal device containing estradiol-17β.

Blood Sampling Schedule	Serum Progesterone Concentration in ng/ml						
	136*	22	148	28	21	7	Mean±S.D.
-24 hr	0.3	0.6	0.2	0.3	0.7	0.5	0.4 ± 0.2
0 hr**	0.8	0.7	0.3	0.3	0.7	0.5	0.6 ± 0.2
4 hr	0.9	0.5	0.3	0.2	0.5	0.5	0.5 ± 0.2
8 hr	0.6	0.7	0.2	0.2	0.3	0.3	0.4 ± 0.2
12 hr	0.3	0.3	0.2	0.2	0.5	0.2	0.3 ± 0.1
16 hr	0.3	0.3	0.2	0.2	0.8	0.7	0.4 ± 0.2
20 hr	0.3	0.4	0.2	0.3	0.4	0.5	0.4 ± 0.1
24 hr	0.3	0.8	0.3	0.3	0.3	0.2	0.4 ± 0.2
28 hr	0.6	0.6	0.4	0.4	0.6	0.6	0.5 ± 0.1
32 hr	0.4	0.5	0.3	0.4	0.7	0.6	0.5 ± 0.1
36 hr	0.3	0.2	0.4	0.3	0.3	0.4	0.3 ± 0.1
48 hr	0.3	0.2	0.4	0.4	0.6	0.6	0.4 ± 0.2
Day 4	0.5	0.6	0.4	0.3	0.7	0.4	0.5 ± 0.1
Day 8	0.8	0.7	0.3	0.2	0.3	0.8	0.5 ± 0.2
Day 12*** 0 hr	0.5	0.2	0.4	0.3	0.2	0.6	0.4 ± 0.2
Day 13 0 hr	0.4	0.4	0.4	0.5	0.5	0.4	0.4 ± 0.01

*Animal No.

continued

**Time of insertion of device.

***Time of removal of device.

Appendix Table 6C. Expt. III. Serum progesterone in ovariectomized heifers treated with intravaginal device containing progesterone + estradiol-17β.

Blood Sampling Schedule	Serum Progesterone Concentration in ng/ml						
	28*	7	22	144	136	148	Mean±S.D.
-24 hr	0.9	0.6	0.3	0.3	0.4	0.5	0.5 ± 0.2
0 hr**	0.8	0.9	0.3	0.6	0.5	0.4	0.6 ± 0.2
4 hr	5.8	6.4	5.4	6.6	6.0	4.2	5.7 ± 0.9
8 hr	4.2	4.2	3.4	4.6	3.6	3.8	4.0 ± 0.4
12 hr	6.4	4.2	4.0	4.2	4.0	4.0	4.5 ± 0.9
16 hr	4.4	5.6	1.8	5.4	4.6	3.0	4.1 ± 1.4
20 hr	2.8	3.2	2.0	5.4	4.0	4.0	3.6 ± 1.2
24 hr	2.8	2.2	1.6	3.2	3.6	4.8	3.0 ± 1.1
28 hr	4.0	3.4	2.2	2.6	3.4	3.6	3.2 ± 0.7
32 hr	3.4	3.2	1.8	3.8	3.8	4.0	3.3 ± 0.8
36 hr	3.2	4.2	1.2	2.2	2.8	3.2	2.8 ± 1.0
48 hr	1.8	1.8	2.2	2.8	2.0	2.2	2.1 ± 0.4
Day 4	1.3	1.2	1.8	1.7	1.1	1.2	1.4 ± 0.3
Day 8	1.8	1.6	1.0	1.5	1.0	1.2	1.4 ± 0.3
Day 12*** 0 hr	1.1	0.9	1.2	0.8	0.7	0.8	0.9 ± 0.2
Day 13 0 hr	0.4	0.5	0.4	0.8	0.6	0.5	0.5 ± 0.1

*Animal No.

**Time of insertion of device.

***Time of removal of device.

Appendix Table 7A. Expt. III. Serum LH in ovariectomized heifers treated with intravaginal device containing progesterone.

Blood Sampling Schedule	Serum LH level in ng/ml						
	148*	21	136	7	22	28	Mean±S.D.
-24 hr	2.7	0.5	2.9	2.1	0.9	5.8	2.5 ± 1.9
0 hr**	3.4	4.9	3.5	4.2	3.9	6.4	4.4 ± 1.1
4 hr	0.7	9.9	1.6	3.0	1.0	6.2	3.7 ± 3.6
8 hr	3.4	1.1	1.8	3.8	0.5	0.8	1.9 ± 1.4
12 hr	8.2	4.8	4.4	1.6	2.9	0.4	3.7 ± 2.8
16 hr	5.8	8.2	3.1	5.0	4.1	0.2	4.4 ± 2.7
20 hr	3.6	5.8	3.3	2.8	5.1	0.2	3.5 ± 1.9
24 hr	7.9	7.9	3.8	6.1	2.5	0.2	4.7 ± 3.1
28 hr	2.3	3.3	4.2	3.7	2.1	0.2	2.6 ± 1.4
32 hr	3.6	2.4	3.8	4.3	2.0	1.4	2.9 ± 1.1
36 hr	3.1	2.2	2.0	7.3	6.1	1.4	3.7 ± 2.4
48 hr	2.3	1.9	3.2	8.6	2.8	1.6	3.4 ± 2.6
Day 4	2.8	3.6	4.7	5.7	5.9	3.0	4.3 ± 1.3
Day 8	3.4	5.5	4.0	2.9	1.4	1.6	3.1 ± 1.5
Day 12*** 0 hr	2.3	2.7	2.9	3.8	3.4	4.5	3.3 ± 0.8
Day 13 0 hr	2.0	2.1	4.9	2.9	4.1	3.3	3.2 ± 1.1

*Animal No.
**Time of insertion of device.
***Time of removal of device.

continued

Appendix Table 7B. Expt. III. Serum LH in ovariectomized heifers treated with intravaginal device containing estradiol-17β.

Blood Sampling Schedule	Serum LH level in ng/ml						
	136*	22	148	28	21	7	Mean ± S.D.
-24 hr	4.5	1.7	3.3	10.0	6.1	4.4	5.0 ± 2.8
0 hr**	9.1	3.0	5.5	11.1	6.6	5.6	6.8 ± 2.9
4 hr	4.2	0.6	1.8	1.2	0.9	1.4	1.7 ± 1.3
8 hr	6.0	6.8	8.5	3.4	6.1	13.2	7.3 ± 3.3
12 hr	3.6	3.2	6.9	9.1	11.2	3.3	6.2 ± 3.4
16 hr	24.2	23.5	27.1	27.3	48.8	45.2	32.7 ± 11.3
20 hr	24.2	27.6	15.5	24.5	11.9	32.8	22.8 ± 7.7
24 hr	6.1	2.1	0.4	3.8	6.8	3.6	3.8 ± 2.4
28 hr	6.8	4.7	5.5	5.7	3.4	1.3	4.6 ± 1.9
32 hr	2.1	3.0	1.4	4.9	4.0	1.5	2.8 ± 1.4
36 hr	3.6	9.8	15.1	7.3	6.5	2.8	7.5 ± 4.5
48 hr	9.9	30.7	42.5	30.3	6.9	1.7	20.3 ± 16.3
Day 4	3.6	43.4	2.7	19.2	23.4	2.6	15.8 ± 16.3
Day 8	1.3	2.9	3.7	4.1	3.4	1.1	2.8 ± 1.6
Day 12*** 0 hr	3.7	2.4	3.6	9.0	21.0	0.2	6.6 ± 7.6
Day 13 0 hr	2.5	1.6	9.4	10.0	8.3	1.7	5.6 ± 4.0

*Animal No.

continued

**Time of insertion of device.

***Time of removal of device.

Appendix Table 7C. Expt. III. Serum LH in ovariectomized heifers treated with intravaginal device containing progesterone + estradiol-17β.

Blood Sampling Schedule	Serum LH level in ng/ml						
	28*	7	22	144	136	148	Mean±S.D.
-24 hr	4.5	0.2	3.7	8.4	4.4	8.0	4.9 ± 3.0
0 hr**	1.6	6.1	2.6	1.2	4.2	4.7	3.4 ± 1.9
4 hr	1.3	1.4	0.2	0.7	0.9	0.9	0.9 ± 0.4
8 hr	4.4	2.6	0.4	1.8	3.2	1.9	2.4 ± 1.4
12 hr	2.1	0.2	2.3	1.0	4.2	1.1	1.8 ± 1.4
16 hr	7.9	0.2	2.6	0.9	3.2	2.8	2.9 ± 2.7
20 hr	0.9	1.6	0.7	0.2	4.3	4.6	2.1 ± 1.9
24 hr	4.8	1.8	0.2	0.2	5.7	1.9	1.9 ± 2.1
28 hr	0.6	2.6	0.2	0.2	0.2	3.7	1.3 ± 1.5
32 hr	1.7	2.6	0.6	0.4	6.8	0.9	2.2 ± 2.4
36 hr	4.9	3.5	0.9	3.5	3.9	2.6	3.2 ± 1.4
48 hr	1.9	7.8	5.3	1.9	6.3	5.4	4.8 ± 2.4
Day 4	6.4	3.4	4.8	9.3	7.5	7.2	6.4 ± 2.1
Day 8	8.3	2.3	1.9	1.9	3.3	7.1	4.1 ± 2.8
Day 12*** 0 hr	5.5	3.1	2.4	2.8	6.3	2.5	3.8 ± 1.7
Day 13 0 hr	2.5	6.5	2.6	4.8	10.4	6.5	5.6 ± 2.9

*Animal No.

**Time of insertion of device.

***Time of removal of device.

Appendix Table 8. Expt. III. Hormone levels in ovariectomized heifers.

a. Analysis of variance for variable Estrogen.

Tests	Source	DF	Sum of Squares	Mean Square	F Value	Prob F
Numerator	Period	2	230.5922	115.29608	6.74830	0.0777
Denominator	Error A	3	51.2556	17.08521		
Numerator	Time	15	8216.6500	547.77667	46.49170	0.0001
Numerator	Period/time	30	530.0790	17.66930	1.49965	0.1072
Denominator	Error B	45	530.2010	11.78225		
Numerator	Treat	2	4008.1086	2004.05431	102.14380	0.0001
Numerator	Period/treat	4	371.6858	92.92144	4.73607	0.0019
Numerator	Time/treat	30	4494.5325	149.81775	7.63600	0.0001
Numerator	Period/time/treat	60	998.9998	16.65000	0.84863	0.7520
Denominator	Error C	96	1883.5133	19.61993		

b. Analysis of variance for variable Progesterone

Numerator	Period	2	9.420625	4.710313	3.17981	0.1817
Denominator	Error A	3	4.443958	1.481319		
Numerator	Time	15	234.741667	15.649444	47.87593	0.0001
Numerator	Period/time	30	21.772708	0.725757	2.22029	0.0076
Denominator	Error B	45	14.709375	0.326875		
Numerator	Treat	2	293.913958	146.956979	477.96285	0.0001
Numerator	Period/treat	4	2.988542	0.747135	2.42998	0.0520
Numerator	Time/treat	30	145.132708	4.837757	15.73432	0.0001
Numerator	Period/time/treat	60	24.814792	0.413580	1.34513	0.0969
Denominator	Error C	96	29.516667	0.307465		

continued

Appendix Table 8. Hormone levels in ovariectomized heifers.
c. Analysis of variance for variable LH.

Tests	Source	DF	Sum of Squares	Mean Square	F Value	Prob F
Numerator	Period	2	1.1919	0.59597	0.01596	0.9859
Denominator	Error A	3	112.0204	37.34014		
Numerator	Time	15	2670.9594	178.06396	9.88366	0.0001
Numerator	Period/time	30	732.2347	24.40782	1.35479	0.1751
Denominator	Error B	45	810.7196	18.01599		
Numerator	Treat	2	2426.7019	1213.35097	69.20787	0.0001
Numerator	Period/treat	4	140.4372	35.10931	2.00259	0.0993
Numerator	Time/treat	30	4504.5381	150.15127	8.56442	0.0001
Numerator	Period/time/treat	60	1578.9128	26.31521	1.50098	0.0374
Denominator	Error C	96	1683.0700	17.53198		

Appendix Table 9A. Expt. IV. Serum progesterone and estradiol-17β in prepuberal heifers treated with control intravaginal device.

Blood Sampling Schedule	Serum Progesterone in ng/ml				Serum Estradiol-17β in pg/ml			
	16*	31	23	Mean ± S.D.	16	31	23	Mean ± S.D.
-24 hr	0.4	0.4	0.4	0.4 ± 0.0	5.6	3.0	5.5	4.7 ± 1.5
0 hr**	0.4	0.4	0.6	0.5 ± 0.1	4.8	4.5	4.2	4.5 ± 0.3
6 hr	0.4	0.4	0.7	0.5 ± 0.2	2.7	4.9	4.6	4.1 ± 1.2
12 hr	0.4	0.5	0.4	0.4 ± 0.1	3.3	4.6	4.4	4.1 ± 0.7
18 hr	0.5	0.4	0.4	0.4 ± 0.1	3.5	5.4	5.1	4.7 ± 1.0
24 hr	0.4	0.7	0.4	0.5 ± 0.2	3.4	3.6	3.1	3.4 ± 0.3
30 hr	0.7	0.5	0.4	0.5 ± 0.2	5.1	5.8	5.3	5.4 ± 0.3
36 hr	0.6	0.5	0.6	0.6 ± 0.1	3.8	6.8	5.9	5.5 ± 1.5
48 hr	0.5	0.5	0.5	0.5 ± 0.0	3.7	6.9	3.7	4.8 ± 1.8
D_4	0.5	0.6		0.5 ± 0.0	3.7	3.6		3.7 ± 0.0
D_8	0.7	0.5		0.6 ± 0.1	4.5	3.2		3.9 ± 0.9
D_{12} 0 hr***	0.6	0.6		0.6 ± 0.0	8.0	4.2		6.1 ± 2.7
D_{12} 6 hr	0.6	0.5		0.5 ± 0.0	3.6	4.3		3.9 ± 0.5
D_{12} 12 hr	0.3	0.2		0.3 ± 0.0	3.6	4.0		3.8 ± 0.3
D_{13}	0.4	0.4		0.4 ± 0.0	4.8	5.3		5.1 ± 0.3
D_{14}	0.6	0.4		0.5 ± 0.1	3.3	3.7		3.5 ± 0.3

*Animal No.

continued

**Time of insertion of device.

***Time of removal of device.

Appendix Table 9B. Expt. IV. Serum progesterone and estradiol-17β in prepuberal heifers treated with intravaginal device containing progesterone + estradiol-17β.

Blood Sampling Schedule	Serum Progesterone in ng/ml					Serum Estradiol-17β in pg/ml				
	10*	29	19	30	Mean±S.D.	10*	29	19	30	Mean±S.D.
-24 hr	0.2	0.6	0.6	0.6	0.5 ± 0.2	5.5	6.6	5.8	6.4	6.1 ± 1.7
0 hr**	0.6	0.6	0.5	0.5	0.6 ± 0.0	5.6	5.3	6.7	3.9	5.4 ± 1.2
6 hr	5.4	4.0	3.6	3.8	4.2 ± 0.8	57.8	44.3	45.0	33.1	45.1 ±10.1
12 hr	2.0	5.6	3.6	3.0	3.6 ± 1.5	25.3	26.0	21.4	14.3	21.8 ± 5.4
18 hr	2.6	2.8	3.6	4.0	3.3 ± 0.7	29.1	24.8	17.5	18.6	22.5 ± 5.4
24 hr	2.5	3.0	2.9	4.5	3.2 ± 0.9	19.0	19.1	13.9	16.2	17.1 ± 2.5
30 hr	2.5	2.9	3.4	3.4	3.1 ± 0.4	17.6	15.3	12.4	22.2	16.9 ± 4.1
36 hr	1.8	3.2	4.5	2.2	2.9 ± 1.2	17.4	12.9	14.6	13.6	14.6 ± 1.9
48 hr	3.2	3.5	3.6	2.1	3.1 ± 0.7	14.9	17.3	16.3	17.6	16.5 ± 1.2
D_4	3.8	2.8			3.3 ± 0.3	6.1	7.6			6.9 ± 1.1
D_8	1.6	1.9			1.8 ± 0.2	7.8	6.7			7.3 ± 0.8
D_{12} 0 hr***	1.8	1.3			1.6 ± 0.3	5.8	5.7			5.8 ± 0.0
D_{12} 6 hr	1.6	1.4			1.5 ± 0.1	3.4	5.0			4.2 ± 1.1
D_{12} 12 hr	0.4	0.9			0.7 ± 0.3	4.3	4.0			4.2 ± 0.2
D_{13} 0 hr	0.5	0.6			0.6 ± 0.0	4.9	5.6			5.3 ± 0.5
D_{14}	0.4	0.6			0.5 ± 0.1	6.2	4.4			5.3 ± 1.3

*Animal No.

continued

**Time of insertion of device.

***Time of removal of device.

Appendix Table 9C. Expt. IV. Serum progesterone and estradiol-17β in prepuberal heifers treated with intravaginal device containing progesterone.

Blood Sampling Schedule	Serum Progesterone in ng/ml					Serum Estradiol-17β in pg/ml				
	15*	26	18	24	Mean±S.D.	15*	26	18	24	Mean±S.D.
-24 hr	0.4	0.2	0.7	0.4	0.4 ± 0.2	5.3	4.0	6.7	4.0	5.0 ± 1.3
0 hr**	0.4	0.6	0.4	0.5	0.5 ± 0.1	4.9	0.4	3.7	3.5	3.1 ± 1.9
6 hr	4.9	5.2	3.0	3.8	4.2 ± 1.0	5.0	5.2	3.8	2.6	4.2 ± 1.2
12 hr	3.8	4.4	2.8	2.6	3.4 ± 0.8	4.8	4.1	3.9	4.0	4.2 ± 0.4
18 hr	2.4	2.4	5.6	4.2	3.7 ± 1.6	3.0	2.1	5.4	3.5	3.5 ± 1.4
24 hr	2.5	2.4	3.6	2.6	2.8 ± 0.6	4.9	0.4	4.3	3.0	3.2 ± 1.9
30 hr	2.7	2.6	2.4	3.0	2.7 ± 0.2	3.2	3.3	3.6	3.5	3.4 ± 0.2
36 hr	1.4	2.0	2.6	2.2	2.1 ± 0.5	3.7	0.4	5.3	3.1	3.1 ± 2.0
48 hr	3.0	3.2	2.4	2.2	2.7 ± 0.5	4.5	0.4	4.0	3.4	3.1 ± 1.8
D_4	2.8	2.6			2.7 ± 0.1	2.7	0.4			1.6 ± 1.6
D_8	1.6	1.6			1.6 ± 0.0	3.9	5.8			4.9 ± 1.3
D_{12} 0 hr***	2.0	3.0			2.5 ± 0.7	5.0	5.0			5.0 ± 0.0
D_{12} 6 hr	1.6	2.4			0.8 ± 0.0	3.9	0.4			2.2 ± 2.5
D_{12} 12 hr	0.8	0.9			0.4 ± 0.1	3.7	0.4			2.1 ± 2.3
D_{13}	0.3	0.5			0.5 ± 0.0	5.3	0.4			2.9 ± 3.5
D_{14}	0.5	0.6			0.5 ± 0.1	3.3	0.4			1.9 ± 2.0

*Animal No.

**Time of insertion of device.

***Time of removal of device.

Appendix Table 10. Expt. IV. Effect of progesterone and progesterone + estradiol-17β therapy on body weight, uterine weight, ovarian weight, adrenal weight and anterior pituitary weight in prepuberal calves.

An. No.	Body Wt. (kg)	Uterine Wt. (g)	Ovarian Wt. (g)	Adrenal Wt. (g)	Ant. Pituitary Wt. (g)
Progesterone Vaginal Device Treatment					
15	188.6	122.2	5.25	10.6	1.01
26	147.7	61.5	3.68	6.2	0.91
18	170.5	40.4	3.31	7.8	0.95
24	163.6	59.5	3.45	9.6	0.87
Mean±S.D.	167.5±16.9	70.9±35.5	3.9±0.8	8.5±1.8	0.94±0.06
Progesterone + Estradiol-17β Device Treatment					
10	175.0	60.9	3.4	6.9	1.01
29	165.9	95.4	3.2	8.6	0.92
19	177.3	84.1	3.2	8.6	0.94
30	136.4	54.6	1.7	7.5	0.79
Mean±S.D.	163.7±18.8	73.8±19.2	2.9±0.8	7.9±0.8	0.92±0.09
Control Vaginal Device Treatment					
16	193.2	45.2	8.7	8.6	0.96
31	161.4	40.1	2.0	6.6	0.89
23	163.6	59.5	3.5	7.6	0.92
Mean±S.D.	172.7±17.8	48.3±10.1	4.7±3.5	7.6±1.0	0.92±0.04

Appendix Table 11A. Expt. IV. Pico moles of ^3H estradiol-17β ($^3HE_2$), ^3H progesterone (^3HP) and ^3H R5020 bound per mg protein of uterine and anterior pituitary tissue at saturation point.

Treatment	An.No.	Uterine Tissue			Anterior Pituitary Tissue		
		$^3HE_2$	^3HP	^3H R5020	$^3HE_2$	^3HP	^3H R5020
Control device	31	5.56	1.50	3.05	0.128	0.042	0.029
	16	6.38	1.84	3.41	0.068	0.058	0.081
	23	5.68	1.35	1.68	0.061	0.048	0.071
	Mean	5.87	1.56	2.71	0.086	0.049	0.077
Progesterone device	15	5.46	1.19	2.65	0.218	0.098	0.183
48 hrs after insertion	26	3.51	0.99	1.75	0.138	0.124	0.240
	Mean	4.49	1.09	2.20	0.178	0.111	0.212
48 hrs after removal	18	1.78	0.18	1.01	0.129	0.098	0.328
	24	3.31	0.58	0.56	0.148	0.138	0.438
	Mean	2.55	0.38	0.79	0.139	0.118	0.383
Progesterone+E_2β device	10	3.36	1.64	3.29	0.178	0.072	0.089
48 hrs after insertion	29	2.34	1.07	1.96	0.128	0.069	0.086
	Mean	2.85	1.36	2.63	0.153	0.070	0.088
48 hrs after removal	19	1.78	0.18	1.01	0.148	0.068	0.099
	30	3.31	0.58	0.56	0.142	0.056	0.082
	Mean	2.55	0.38	0.79	0.145	0.062	0.091

continued

Appendix 11B. Expt. IV. Pico moles of ^3H estradiol-17β ($^3HE_2$), ^3H progesterone (^3HP) and ^3H R5020 bound per mg protein of posterior hypothalamus and anterior hypothalamus tissue at saturation point.

Treatment	An.No.	Posterior Hypothalamus			Anterior Hypothalamus		
		$^3HE_2$	^3HP	^3H R5020	$^3HE_2$	^3HP	^3H R5020
Control device	31	0.113	0.056	0.338	0.081		
	16	0.104	0.052	0.156	0.136		
	23	0.119	0.079	0.201	0.041		
	Mean	0.112	0.062	0.232	0.086		
Progesterone device	15	0.175	0.058	0.771	0.293		
48 hrs after insertion	26	0.078	0.162	0.638	0.168		
	Mean	0.127	0.110	0.705	0.231		
48 hrs after removal	18	0.089	0.055	0.678	0.162		
	24	0.158	0.168	0.435	0.095		
	Mean	0.124	0.112	0.557	0.129		
Progesterone+E_2β device	10	0.194	0.097	0.517	0.261		
48 hrs after insertion	29	0.142	0.122	0.522	0.361		
	Mean	0.168	0.109	0.519	0.311		
48 hrs after removal	19	0.259	0.271	0.428	0.178		
	30	0.182	0.083	0.618	0.053		
	Mean	0.221	0.177	0.523	0.116		

Non-Detectable

Non-Detectable