PROGESTERONE AND LH SECRETION AND ESTROUS CYCLE ALTERATIONS FOLLOWING ENDOMETRIAL IRRITATION IN COWS

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ABSTRACT

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By

Bradley Edson Seguin[II]

Intrauterine infusion of dilute iodine solution (DIS) was used to alter estrous cycle lengths in cows. During these altered cycles, blood serum progesterone and LH levels were quantified and the effect of DIS on the epithelium of the uterus was studied by endometrial biopsy. In a second experiment the effect of intrauterine administration of a luteolysin, prostaglandin $F_{2\alpha}$, 24 hours after infusion of the iodine solution was investigated.

In the first experiment 31 dairy cows, 30 to 60 ($\bar{x} = 40.7 \pm 1.8$) days postpartum, were randomly assigned to one of four groups. Cows in their respective groups were (1) infused during estrus (day 0), (2) infused during the early luteal phase (day 4) of the estrous cycle, (3) infused during the late luteal phase (day 15) of the estrous cycle, or (4) noninfused controls. Cows were infused with 255 ml. of DIS (I:KI:saline = 1:2:1020). Endometrial biopsies and serum samples were taken during the treated cycles in all four groups. The endometrial sections were prepared for histologic evaluation and serum samples from five cows per group were assayed for LH and progesterone.

Cycle lengths of cows infused during estrus averaged 22.4 ± 0.1 days and did not differ ($P>0.05$) from cycles in control cows which

averaged 21.2 ± 0.7 days. Infusion on day 4 of the estrous cycle shortened treated cycles to 10.6 ± 0.2 days ($P<0.01$). Infusion on day 16 lengthened treated cycles to 25.1 ± 0.6 days ($P<0.01$). Subsequent cycles were of normal length for all groups ($P>0.05$). Despite the alteration in cycle lengths, the characteristic progesterone decline and LH peak of the proestrous period were observed in the pooled curves for each group. Considering only the 3-day period prior to estrus for each group, the curves for serum progesterone and LH were similar. These results indicate that DIS infusion affects cycle lengths by altering only the length of the luteal phase of the estrous cycle.

On histopathologic examination 24 hours after infusion, the surface epithelium of the uterus was either destroyed or severely distorted. Marked swelling, vacuolization and destruction of cells in the superficial endometrium with some infiltration of inflammatory cells were observed at this time. Regeneration of surface epithelium and repair of the superficial endometrium appeared to be complete in nearly all cases by the time of estrus 7 to 10 days following infusion. Following this experiment, days open were greater ($P<0.01$) in the three groups which received DIS infusion, due to post-service anestrus in five cows, than in the control group. The interval from first service to conception and the services per conception did not differ ($P>0.05$) between the four groups. Although the cause of the post-service anestrus following infusion of DIS was not determined, the infusion of DIS may have adversely affected the subsequent reproductive performance of these cows.

In view of the degree of endometrial damage present following infusion of DIS, destruction of the endometrial luteolytic factor was suspected and a second experiment initiated. Following DIS infusion on day 15, 5 mg. of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$ THAM salt), a proposed uterine

luteolytic substance, was infused intrauterinally in five cows on day 16. Estrus and ovulation in these cows occurred on days 19 and 20, respectively. The ability of $PGF_{2\alpha}$ to overcome the cycle lengthening tendency of DIS infusion on day 15 of the estrous cycle (19 versus 25 day cycles) is consistent with, but not proof of, the proposal of $PGF_{2\alpha}$ being the uterine luteolytic factor.

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Bradley Edson Seguin[II]

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INTRODUCTION

Maximum reproductive efficiency is one of the major concerns of modern cattlemen in maintaining a profitable beef or dairy cattle operation. Effective heat detection is probably the single most important factor in attaining maximum herd reproductive performance where artificial insemination is used. Proper heat detection requires a substantial effort in terms of time and labor input and, all too frequently, is the first area neglected. An effective estrous synchronization program for cattle would have obvious labor-saving advantages for animal agriculture and the A.I. industry. Estrous synchronization is not a new idea; in fact, programs and products have been developed for this purpose in the past, but all have shortcomings which presently make them unsatisfactory for practical application. Development of estrous synchronization procedures and other forms of fertility control are dependent upon knowledge of and ability to control the normal events of the estrous cycle.

The major effort in estrous synchronization work to date has been with the use of progestins which prevent the onset of estrus by maintaining the CL. Behavioral estrus is well synchronized following withdrawal of the progestin but fertility at this synchronized estrus has been unsatisfactory.

Utero-ovarian interrelationships play an important role in the regression of the CL at the end of the normal estrous cycle. Stimulation of the natural luteolytic mechanism or administration of a

luteolytic substance to reproduce the physiologic regression of the CL may prove to be an effective method of estrous synchronization. Endometrial irritation, caused for example by intrauterine infusion of DIS, is capable of altering the length of the estrous cycle presumably by affecting the uterine luteolytic factor.

Endometritis and pyometra are two common veterinary problems in the cow which alter the estrous cycle. Endometritis is frequently characterized by short irregular cycles presumably due to excessive stimulation of the uterine luteolytic mechanism. Pyometra, a more severe condition with accumulation of quantities of purulent exudate in the uterus, is characterized by anestrus and persistence of the CL. The accumulation of exudate and the extensive nature of the pyometra condition may be destroying the luteolytic mechanism of the uterus.

Recent development of sufficiently sensitive techniques to quantify hormones in physiologic concentrations has enabled investigators to describe the endocrine status of the cow during the estrous cycle and pregnancy. This information will provide a basis for further research in the area of bovine reproductive physiology.

The objective of this project was to study utero-ovarian interaction and more specifically the endocrine response and alteration of the estrous cycle following endometrial irritation caused by the intrauterine infusion of DIS.

REVIEW OF LITERATURE

Loeb (1923, 1927) was the first to report that hysterectomy in guinea pigs inhibited the onset of estrus and greatly prolonged the life span of the corpora lutea. He proposed that "an internal secretion of the uterine mucosa might have a specific abbreviating effect upon the corpus luteum." In recent years the role of the uterus in CL regression has received high priority from researchers and reviews of the subject have been presented by Ginther (1966, 1967, 1968a, 1968b), Melampy and Anderson (1968), Anderson, Bland and Melampy (1969), Schomberg (1969) and McCracken (1972). While a uterine luteolytic substance has not been positively isolated and identified, the experimental evidence suggests that such a substance exists.

Utero-Ovarian Interaction

Wiltbank and Casida (1956) were the first to apply Loeb's techniques to farm animals and found that total hysterectomy also prevents CL regression in cows and ewes. Anderson, Neal and Melampy (1962) similarly found that total hysterectomy in heifers during the luteal phase of the cycle prevents behavioral estrus and CL regression for at least 270 days. Following partial removal of one or both uterine horns normal CL regression and estrus occurred indicating that only part of each uterine horn was needed to stimulate CL regression. Similar results following total hysterectomy have been reported in gilts by du Mesnil du Buisson and Dauzier (1959) and Spies *et al.* (1960) and in

mares by Ginther (1971). Therefore, Loeb's suggestion of the presence of a uterine luteolytic factor in guinea pigs is also appropriate for these farm animals.

A unilateral effect of hysterectomy has been shown in sheep by Inskeep and Butcher (1966) and Moor and Rowson (1966) and in cattle by Ginther (1967). Unilateral hysterectomy caused maintenance of the CL located in the adjacent ovary, but the CL in the opposite ovary connected to the remaining uterine horn regressed normally. Similar observations in cattle, sheep and guinea pigs with congenital absence of one uterine horn by Spriggs (1946), McCracken and Caldwell (1969) and Bland (1970), respectively, and in heifers with the absence of uterine glands as cited by Ginther (1968b) have eliminated surgery per se as the causative factor in the local luteolytic effect of the uterus.

The method of utero-ovarian communication appears to be a local humoral interaction. As reviewed by McCracken, Baird and Goding (1971), studies involving vascular autotransplantation of various parts of the reproductive tract to the neck of sheep have confirmed that the normal spatial and vascular relationships between an ovary and its adjacent uterine horn must be maintained for normal luteal regression to occur. This would indicate that the utero-ovarian interaction is, at least partly, a local one and independent of neural mechanisms. Since the luteolytic factor appears to have only a weak systemic effect, a more direct pathway from the uterus to the ovary must be present. Barrett *et al.* (1971) reported that surgical separation of the utero-ovarian vein and ovarian artery prevented CL regression in sheep. Ginther (1968b) reported that no vascular shunts have been found between these vessels. This suggests a counter current vascular pathway for utero-ovarian communication involving the diffusion of a luteolytic factor

from the utero-ovarian vein to the ovarian artery. Infusion of labelled $PGF_{2\alpha}$ into the uterine vein resulted in a much greater concentration (5- to 6-fold increase) of radioactivity in ovarian arterial blood than in iliac arterial blood which indicated to McCracken *et al.* (1971) that transport of $PGF_{2\alpha}$ via a counter current mechanism could occur. Conley and Hawk (1970) reported removal, ligation or occlusion of the oviducts in ewes or salpingectomy in cows did not alter estrous cycle length, indicating that utero-ovarian transfer of the luteolytic factor does not depend upon functional oviducts. Transfer of a uterine luteolytic factor from the uterus to the ovaries via lymphatic vessels is another possibility which has not been thoroughly investigated.

Early regression of the CL also occurs in progesterone-treated and oxytocin-treated intact and unilaterally hysterectomized cows as reported by Woody and Ginther (1968) and by Ginther et al. (1967), respectively, but only when the retained horn is adjacent to the ovary containing the CL. Daily injections of oxytocin during the first week of the estrous cycle by Armstrong and Hansel (1959) inhibited luteal development and produced 7- to 10-day cycles. However, daily injections of oxytocin starting on day 15 of the cycle did not affect the normal onset of estrus at day 20 to 21. Anderson, Bowerman and Melampy (1965) reported that the CL was maintained and estrus did not occur in heifers given oxytocin for 7 days beginning on day 1 and hysterectomized on day 2. Ginther *et al.* (1967) reported that oxytocin administered daily from day 3 to day 7 or 8 produced shortened estrous cycles in intact heifers. In unilaterally hysterectomized heifers treated in the same manner the estrous cycle was shortened only if the remaining horn was adjacent to the CL. Woody and Ginther (1968) reported that daily progesterone injections from day 1 through day 10 in intact heifers shortened the

estrous cycle to 16.4 days. In unilaterally hysterectomized heifers, the same progesterone treatment similarly shortened the estrous cycle if the remaining horn was adjacent to the CL but not if the remaining horn was opposite the CL. The mechanism of action involved with oxytocin and progesterone administration is not known, but it is clear that local utero-ovarian interaction is at least partially involved.

Wiltbank (1966) reported that a single injection of 5 mg. of estradiol valerate caused early CL regression in heifers during the early and mid-luteal phases of the estrous cycle. Regression of the CL, measured by progesterone content of the CL, occurred in the presence of a cycling or pregnant uterus or in the absence of the uterus. However, the luteolytic action of estrogen was not as marked in hysterectomized heifers as in intact open or pregnant heifers. The same treatment at day 15 or 16 caused a high incidence of cystic ovarian follicles. The luteolytic effect of estrogen was reversed by concurrent administration of human chorionic gonadotropin (HCG). Wiltbank (1966) suggested that estrogen may cause decreased gonadotropin levels, primarily LH, resulting in regression of the CL. Similarly, Brunner, Donaldson and Hansel (1969) reported that 5 mg per day of estradiol-17β for 6 or 15 days caused only partial luteal regression in hysterectomized heifers, while similar doses caused essentially complete luteolysis in intact heifers. These results suggest that a uterine luteolytic factor may be required in addition to the anti-luteotropic effect of estrogen for complete luteolysis.

Endometrial Irritation and Uterine Distention

Endometrial irritation and uterine distention have been used as experimental techniques to study utero-ovarian interaction and as clinical treatments for anestrus in cows and mares. Hignett (1940) and

Hancock (1948) reported the intrauterine infusion of aqueous iodine solution as a successful clinical treatment for the anestrous condition in heifers. The majority of Hancock's heifers (9 of 11) were in heat on the 7th day following infusion. Palpation *per rectum* indicated these induced estrous periods were ovulatory and subsequent estrous periods occurred at 21-day intervals. Hancock (1948) stated that uterine distention rather than irritation was the probable mechanism of action. Gripper (1969) used uterine irrigation with 200 ml of a 20% calcium borogluconate solution to treat anestrous dairy cows. In 36 of 50 cows treated, estrus occurred an average of 6.5 days after treatment. Roberts (1971) reported that the intrauterine infusion of 250 to 500 ml of warm physiologic saline was a common treatment for anestrous mares. Estrus usually occurred 2 to 4 days following treatment.

Intrauterine infusion of a gel-like substance by Yamauchi *et al.* (1967) or a dilute iodine solution by Morrow *et al.* (1971) and Nakahara, Domeki and Yamauchi (1971b) during the early luteal phase shortened the estrous cycle. Yamauchi *et al.* (1967) reported that intrauterine infusion of a gel-like substance during the early luteal phase in 13 of 14 cows resulted in a 12.5-day cycle while late luteal phase infusion of 5 cows resulted in a 25.8-day cycle. The interval from infusion to estrus was quite consistent with estrus occurring 9.3 ± 1.6 days after infusion. Similar treatment during metestrus, mid-luteal phase or proestrus did not alter cycle length. Signs of a temporary endometritis were observed following intrauterine infusion of this substance.

Morrow *et al.* (1971) reported 10.3-day cycles following infusion of dilute iodine solution on day 4 but no alteration after infusion on day 1 or day 11. Intrauterine infusion of the same volume (250 ml) of sterile saline did not alter cycle length indicating that uterine

irritation and not uterine distention was the probable stimulus involved. Nakahara *et al.* (1971b) infused cows with 20 to 40 ml of dilute iodine solution at all stages of the estrous cycle (Table 1).

Table 1. Modification in estrous cycle length of cows by intrauterine injection of iodine solution*

Day** of Infusion	No. of cows	Mean Treated Cycle Length	Mean Days from Treatment to Ovulation
0-1	4	21.5	21.0
2-3	4	11.5	9.0
5-7	4	15.3	9.3
9	2	19.0	10.0
13	4	22.8	10.0
15	5	24.4	9.4
16	4	24.0	8.0
17	4	23.3	6.0
19	3	22.3	3.3

*Nakahara *et al.*, 1971b.

**Day 0: day of ovulation.

Again the interval from treatment to ovulation was closely grouped when cows were treated between day 2-3 and day 16 and provided encouragement for further investigation of this technique for estrous synchronization.

Grunert, Schultz and Esser (1971) reported infusion of an iodine solution from day 13 to day 17 extended the estrous cycle by 1 to 4 days but infusion before day 12 had no effect on cycle length. This difference from the previously discussed reports has not been explained.

Relatively severe epithelial and endometrial damage was present 24 hours after infusion and epithelial regeneration was complete by the 5th day after infusion. Intrauterine infusion of 40 ml of 0.2% nitrofurazone solution on days 1, 2 or 3 of the estrous cycle has been investigated by Ginther and Meckley (1972). Infusion on day 1 or day 2 had no effect on cycle length; however, infusion on day 3 shortened the subsequent diestrous period by 5.5 days with estrus occurring at day 15.5.

Infusion of small volumes of iodine solution (0.1 to 5.0 ml) in cows as reported by Nakahara, Domeki and Yamauchi (1971a) or alcohol solution (0.05 ml) in sheep as reported by Woody, Ginther and Pope (1969) was capable of altering cycle lengths as previously discussed which, along with the work of Morrow *et al.* (1971), eliminates uterine distention as the responsible mechanism of action. Woody *et al.* (1969) and Nakahara *et al.* (1971a) reported that volumes < 1.0 ml caused local endometrial damage at the site of deposition and were effective in altering cycle lengths only when placed in the horn adjacent to the CL. Five milliliters of solution caused endometrial damage in both horns and did affect cycle length regardless of where the CL was located.

Induced bacterial or viral endometritis is also capable of altering estrous cycle lengths. Kendrick and McEntee (1967) reported on 12 heifers bred with semen containing IBR-IPV virus and 10 of 12 heifers returned to estrus in 11 to 15 days. Necrotizing endometritis was present in the heifers with short cycles. Lynn, McNutt and Casida (1966) reported that induced bacterial infection in the early postpartum period caused persistence of the CL in cows. Ginther (1968b) described clinical cases of pyometra in the bovine as being characterized by a persistent CL also. Raw semen containing sediment from centrifuged seminal fluid was inoculated into the uteri of 16 heifers

at estrus by Hansel and Wagner (1960) and 9 of the 16 cycles were shortened, ranging from 6 to 13 days in length. The remaining 7 cycles were of normal length (20 to 22 days). *Vibrio fetus* organisms were known to be present in some of the inoculations and all heifers with short cycles displayed purulent vaginal discharges prior to the precocious estrus. Brinsfield and Hawk (1968) infused ewes with bacterial cultures the day after estrus and found that CL formation was inhibited through day 6. Ginther (1968b) described clinical cases of endometritis in the bovine as being characterized by shortened, irregular estrous cycles.

Hawk (1968) reviewed the effects of intrauterine devices (IUD) on CL function. Insertion of an IUD in sheep or cattle during the early luteal phase resulted in shortened cycles of approximately 12 days. A local utero-ovarian mechanism is indicated by the fact that the IUD can cause early CL regression if placed in the cranial tip of the horn adjacent to the CL but not if placed in the horn opposite the CL. The mechanism of action for the IUD is not known but may involve uterine distention or irritation.

These experiments suggest that the uterus may produce a luteolytic substance which is, at least partially, locally active. Ginther (1968a) postulated that

> "the bovine uterus terminates the life of the corpus luteum by means of a uterine luteolytic mechanism which acts through two pathways: 1) a systemic utero-pituitary-ovarian pathway involving altered circulating levels of a pituitary luteotropin, and 2) a local utero-ovarian pathway involving the local transport of a uterine luteolysin directly from a uterine horn to the adjacent ovary. During pregnancy the uterine luteolytic mechanism is inhibited by the embryo, and regression of the corpus luteum is prevented."

Luteolytic Factor

As first suggested by Loeb (1923, 1927) and supported by subsequent research, the presence of a uterine luteolytic factor capable of causing the demise of the CL has been established although not identified. Caldwell and Moor (1971) demonstrated the presence of a luteolytic factor in uterine vein blood during the late luteal phase (day 14) of the estrous cycle in sheep. Late luteal phase blood, when infused into the ovarian artery of early luteal phase (day 8) ewes, caused CL regression and shortened estrous cycles (10 to 11 days versus the normal 16-day cycles). Blood from the jugular vein of late luteal phase ewes or from the uterine vein of day 8 ewes had no effect on the CL or cycle length. Reports by Williams *et al.* (1967) and Lukaszewaska and Hansel (1970) involving endometrial extracts from late luteal phase cows have indicated the presence of an endometrial luteolytic factor when these preparations were tested in pseudopregnant rabbits and hamsters. Attempts to date by these and other workers to isolate and identify this luteolytic factor have not been successful.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which McCracken (1972) and others have proposed as being a uterine luteolytic factor, was found to be abundant in the menstrual fluid of women by Pickles (1967) and in the uterus of sheep by Wilson *et al.* (1972b). Wilson *et al.* (1967b) found that endometiral $PGF_{2\alpha}$ in ewes was significantly higher at day 14 of the estrous cycle than at days 3, 5 or 11. McCracken *et al.* (1971) reported a 10-fold increase, from 2 ng/ml to about 25 ng/ml, in the concentration of $PGF_{2\alpha}$ in uterine venous plasma of ewes at the time of luteal regression. Premature luteal regression was induced in sheep by infusion of $PGF_{2\alpha}$ into the uterine vein adjacent to the ovary containing the CL by McCracken *et al.* (1971). In this same report infusion of $PGF_{2\alpha}$ into

the systemic circulation had no effect. Louis, Hafs and Morrow (1972) and Rowson, Trevit and Brand (1972) reported intrauterine infusion of $PGF_{2\alpha}$ in luteal phase cows caused premature luteal regression and estrus in about 3 days. Rowson *et al.* (1972) found the same treatment ineffective from day 1 through day 4 of the bovine estrous cycle.

The mechanism of action of $PGF_{2\alpha}$ is not known although DuCharme, Weeks and Montgomery (1968) found $PGF_{2\alpha}$ to be a strong venoconstrictor in dogs and cats. Venoconstriction may cause the CL to succumb to conditions such as limited substrate availability or accumulation of metabolites.

The report by Wilson, Butcher and Inskeep (1972a) of pregnant ewes having higher endometrial levels of $PGF_{2\alpha}$ than nonpregnant ewes on days 15 and 16 of the cycle may be contradictory to the proposal of $PGF_{2\alpha}$ being the uterine luteolytic factor.

Serum Progesterone and LH During the Bovine Estrous Cycle

Several authors including Shemesh, Ayalon and Lindner (1968); Stabenfeldt, Ewing and McDonald (1969); Pope, Gupta and Munro (1969); Hansel and Snook (1970); Henricks, Dickey and Niswender (1970); Lamond *et al.* (1971); Swanson, Hafs and Morrow (1972); and Wettemann *et al.* (1972) have reported serum or plasma progesterone concentrations during the bovine estrous cycle. To summarize these reports, serum or plasma progesterone is low (< 1 ng/ml) from estrus to about day 5, rises slowly to a 5- to 6-fold increase (5 to 7 ng/ml) at days 15 to 17 and then drops sharply to low levels (< 1 ng/ml) 1 to 4 days before the onset of estrus.

Peripheral blood plasma or serum LH levels for the cow reported by Hansel and Snook (1970); Henricks *et al.* (1970); Swanson and Hafs (1971); Swanson *et al.* (1972); and Wettemann *et al.* (1972) were low (< 1 ng/ml)

during the major portion of the estrous cycle and rose to a sharp 10- to 20-fold peak near the onset of estrus. Duration of this LH peak has been reported to be 6 to 10 hours by Henricks *et al.* (1970) and Swanson and Hafs (1971).

In altered cycles as previously discussed, peripheral blood levels of progesterone or LH have rarely been reported. Louis *et al.* (1972) reported an LH peak (7.3 ng/ml) about 70 hours or near the onset of estrus following intrauterine infusion of $PGF_{2\alpha}$ in luteal phase cows.

Histology of the Bovine Endometrium

Johnson (1965) summarized the normal anatomical landmarks of the wall of the bovine uterus. The perimetrium is the thin, outer serous surface of the uterus. The myometrium is the middle layer composed of smooth muscle fibers, connective tissue, stroma and surface epithelium. The endometrium is the innermost layer and is composed of mucous glands, connective tissue, stroma and surface epithelium. The endometrial layer can be further separated as described by Skjerven (1956) into (1) pseudostratified columnar epithelium with an attached basement membrane, (2) the stratum compactum which is a narrow zone of densely cellular connective tissue just beneath the basement membrane and (3) the stratum spongiosum, a more loosely arranged layer of collagenous connective tissue extending to the myometrium.

Skjerven (1956) considered the risks involved to the animal in connection with the biopsy examination and the histologic condition of the endometrium during the various stages of the estrous cycle in reproductively normal cows. The introduction of the instrument through the cervix and the actual removal of the endometrial sample did not appear to cause the animal pain, nor did serious hemorrhage or perforation of

the uterine wall occur. Skjerven (1956) reported a 57.8% first service conception rate for all animals submitted to the endometrial biopsy procedure. Of 25 procedures carried out between day 7 and day 13 after insemination, 13 or 52% were taken from animals which later proved to have conceived. No deviation from normal was detected in calves born following exposure to endometrial biopsy. In reproductively normal animals, neutrophils occurred in the superficial stroma and surface epithelium in varying numbers at and near estrus, but at other stages of the cycle they were practically absent. The numbers of eosinophils varied greatly, appearently without relation to known biological factors. Lymphocytic cells appeared to be only scattered (not clustered in clones) in biopsies from reproductively normal animals. The number of plasma cells was very low in heifers and monoparous cows and increased with the increasing age of the animal. Mast cells were always commonly present in the stratum compactum and were most plentiful during the follicular phase of the estrous cycle.

Simon and McNutt (1957) reported that endometrial sections taken from repeat breeder cows did not differ from sections taken from apparently normal virgin heifers. De Bois (1961) monitored uterine involution in the postpartum cow by palpation *per rectum*, bacterial culture and endometrial biopsy. The agreement between these 3 techniques on the status of the uterine environment was variable. De Bois (1961) stated that endometrial biopsy was the superior method of examination even though endometritis, as diagnosed by endometrial biopsy, was poorly correlated with breeding efficiency.

MATERIALS AND METHODS

An experiment was designed to study the effects of cycle alteration by infusion of dilute iodine solution (DIS) on serum progesterone and LH levels in cows. The histologic response of the uterus to this treatment was also investigated. In an additional experiment the effects of $PGF_{2\alpha}$ infusion on peripheral blood levels of progesterone and LH after the endometrium had been damaged by DIS infusion were investigated.

Animals, Experimental Design and Endometrial Biopsies

Thirty-one lactating dairy cows, 30 to 60 days postpartum, were randomly assigned to one of four groups. Cows in their respective groups were (1) infused during estrus (day 0), (2) infused during the early luteal phase (day 4) of the estrous cycle, (3) infused in the late luteal phase (day 15) of the cycle or (4) noninfused controls. The infusion, 5 ml of strong iodine solution[1] in 250 ml of saline (I:KI:saline = 1:2:1020), was administered via infusion pipette into the body of the uterus. Dunnett's procedure as described by Steel and Torrie (1960) was used to compare cycle lengths in cows infused with DIS against cycles in control cows.

Endometrial tissue was taken for histologic examination during treated cycles in all four groups, as outlined in Table 2.

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[1]Lugol's Solution^{R} (strong iodine solution), Humco Laboratory, Texarkana, Texas.

Table 2. Schedule for endometrial sampling

Time of Infusion	Days Sampled
Estrus (day 0)	Estrus, day 1, estrus
Day 4	Estrus, day 4, day 5, estrus
Day 15	Estrus, day 15, day 16, estrus
Controls	Estrus, estrus

A technique similar to that described by Skjerven (1956) was used to obtain the endometrial tissue samples. A Yoeman rectal biopsy forceps[2] was passed into the body of the uterus through the vagina and cervix and the sample of endometrium clipped from the area of the junction of the horns and the body of the uterus. Tissue sections were fixed in 10% formalin, embedded in paraffin and stained with hematoxylin and eosin stain. The sections were pooled by day within each group for evaluation.

A second experiment was designed as a result of the first to determine if $PGF_{2\alpha}$ would cause luteolysis after the endometrium had been damaged by infusion of DIS. Five cycling nonlactating dairy cows were infused with 255 ml of DIS on day 15 of the estrous cycle as previously described in the first experiment. On day 16, 5 mg of $PGF_{2\alpha}$[3] in 1.0 ml of saline was infused into the uterus of these same cows. Cycle lengths and progesterone and LH secretion in these cows were compared with the Group III animals in the first experiment which were infused with DIS on day 15.

[2]The Lawton Company, Inc., 200 Anderson Ave., Moonachi, N.J. 07074.

[3]Dr. James Lauderdale, The Upjohn Company, Kalamazoo, Mich.

Blood Samples

In the first experiment 40 ml of blood were collected from the jugular vein during the treated cycles on days 0, 2, 4, 7, 8, 9, 10, 11, 12, 15, 18 and daily through the estrous period following infusion. Blood serum was harvested and stored at -15 C. until assayed.

In the second experiment 40 ml of blood were collected from the jugular vein on days 11, 15, 16, 17 and then twice daily through estrus. Blood plasma was harvested and stored at -15 C. until assayed.

LH and Progesterone Assays

The double antibody radioimmunoassay (RIA) for LH reported by Oxender, Hafs and Edgerton (1972) was used to quantify LH in all samples in dilution duplicates.

In the first experiment serum progesterone was measured by competitive protein binding as described by Smith *et al.* (1972) and modified from the method described by Murphy (1965). Approximately 2000 dpm of ^{3}H-protesterone[4] (specific activity = 50 c/mM) in 10 µl ethanol was added to 1.0 ml serum and progestins were extracted twice with 5 ml freshly redistilled trimethyl pentane by mixing on a vortex mixer at maximum speed for 2 min. The combined trimethyl pentane was evaporated under nitrogen to approximately 3 ml and radioactivity in 0.5 ml was determined in a liquid scintillation spectrometer (Nuclear Chicago Model Mark I) to estimate procedural losses of progesterone.

Aliquants (0.5 to 1.0 ml) of the trimethyl pentane extract were assayed for total progestin. Dog plasma (Colorado Serum Company, Denver) was the source of binding protein. Endogenous steroids were adsorbed

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[4]Obtained from New England Nuclear, Boston, Mass.

from 2.5% dog plasma in sterile water (Cutter Laboratories, Inc., Berkeley, California) by stirring for 30 min. with 8 gm Florisil (30 to 60 mesh, Matheson, Coleman and Bell, Cincinnati, Ohio) per 100 ml. Depending upon titer, each batch of dog plasma was further diluted to 0.67% and approximately 8000 dpm ^{3}H-corticosterone (specific activity = 45.3 c/mM) per ml was added as the competitor in the progestin assay. Dog plasma (1.5 ml) was added to tubes containing standard progesterone or unknown steroid. The mixture was vortex-mixed for 15 sec., then incubated at 4 C. for 8 to 24 hours. Thereafter, 80 mg of Florisil was added and the contents of each tube were immediately vortex-mixed for 45 sec. After the Florisil settled, 0.5 ml of supernatant fluid was transferred to a scintillation vial with 10 ml of Bray's scintillation fluid (Bray, 1960). Radioactivity was quantified by linear interpolation between progesterone standards (0, 0.1, 0.5, 1.0, 1.5, 2.0, 5.0 and 10.0 ng). Except for the scintillation vials, all glassware was rinsed with 5% trimethylchlorosilane (Sargent Welch Scientific Co., Detroit, Mich.) in toluene and dried prior to use.

In the second experiment plasma progesterone was determined by the RIA developed and validated by Kittok (1972), as follows:

All glassware is carefully washed 4X with detergent and rinsed 8X with tap water, 5X with distilled water, 3X with glass distilled water, and 3X with analytical grade acetone, and allowed to air dry. Then the glassware is rinsed with a solution of 5% chlorotrimethylsilane in toluene and air dried.

To each 0.5-ml serum sample, in a 15-ml culture tube fitted with screw cap with teflon liner, 3,000 dpm of ^{3}H-1,2-progesterone (New England Nuclear; 34 c/mM; repurified by column chromatography) is added. The contents are vortexed and equilibrated for 30 min. The serum and tracer are then mixed by gentle inversion with 10 ml of benzene:hexane (1:2, V:V, both monograde) for 20 min. The tubes are then stored at -20 C. for at least 1 hour to freeze the aqueous phase, and the solvent extract is decanted into conical tubes and evaporated under nitrogen to approximately 2.5 ml. A 0.5-ml aliquant is taken to measure procedural losses and 0.25- and 0.50-ml aliquants are placed in disposable culture tubes (12X75 mm) for radioimmunoassay.

Two sets of standard progesterone (Sigma Chemical Co.), 0.0, 0.1, 0.5, 1.0, 1.5, 2.0, and 5.0 ng, dissolved in redistilled absolute ethanol are included in each assay. Standards and unknowns are dried under nitrogen. Antibody[5] (0.2 ml) diluted 1:3000 in 0.1% gelatin (Knox Gelatin, Inc.) in 0.1 M phosphate buffered saline, is added to each tube, vortexed for 10 sec. and allowed to incubate at room temperature for 30 min. Two hundred microliters of 0.1% gelatin in phosphate buffered saline, containing 30,000 cpm of ^{3}H-1,2-progesterone (New England Nuclear), is added to each tube. The tubes are vortexed for 5 sec. and incubated at 5 C. for 12-18 hr. Hereafter, the tubes always remain refrigerated.

To separate the bound and unbound progesterone, 1 ml of 0.025% Dextran 150 (Pharmacia, Uppsala, Sweden) and 0.25% carbon decolorizing neutral norit (Fisher Scientific Co.) in glass distilled water is added to each tube. After vortexing and a 10-min. incubation in an ice bath, the tubes are centrifuged at 2500 xg for 10 min. at 5 C. A 0.5 ml aliquant of the supernatant liquid is diluted with Bray's Solution (1960) and the radioactivity is quantified in a liquid scintillation spectrometer (Nuclear Chicago Corp., Mark I).

For comparison among assays, a standard serum and the extract from a blank extraction tube (treated as a serum sample) are assayed with each set of unknown samples.

Progesterone was estimated in 16 serum unknowns by (1) the RIA described above, (2) by competitive protein binding assay of progesterone isolated from LH-20 Sephadex columns, and by RIA of progesterone isolated from LH-20 Sephadex columns.

Estimations by radioimmunoassay of benzene:hexane extracts (2.9 $\pm$ .70 ng/ml) did not differ significantly from those by radioimmunoassay of eluate from LH-20 Sephadex columns (2.7 $\pm$ .80 ng/ml) ($r = 0.94$) or from those by competitive protein binding assay of eluate from columns (3.9 $\pm$ 1.35 ng/ml) ($r = 0.78$).

Serum samples from 5 cows per group were assayed for LH and progesterone. Results were analyzed by the split plot method of Gill and Hafs (1971) for repeated measurements on animals and the appropriate curves constructed. The data were plotted from estrus through day 15 for groups I, III and IV and through day 7 for group II. The remaining data were plotted on a minus-day basis from the estrus following DIS infusion to day 15 or day 7, respectively.

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[5]Anti-progesterone, generously supplied by G. D. Niswender, Department of Pathology, The University of Michigan, Ann Arbor. The rabbit antiserum (#869) was prepared against 6β-Succinyl progesterone conjugated to bovine serum albumin.

Estrous Detection and Palpation

In both experiments cows were observed at least twice daily for signs of estrus. Cows were palpated *per rectum* at least twice weekly to confirm the presence of a CL at the time of infusion and to follow CL regression, ovulation and subsequent CL formation. These observations were also conducted during at least one cycle before and after the treated cycle. Subsequent reproductive performance of cows on the DIS infusion experiment was recorded and summarized. No attempt was made to rebreed the five cows used in the second experiment.

RESULTS AND DISCUSSION

The interval from parturition to the start of the treated cycle in the first experiment averaged 40.7 ± 1.8 days (Table 3) and did not differ between groups (P>0.05).

Table 3. DIS infusion study, postpartum interval to treated cycle

Treatment	No.	Postpartum Interval to Treated Cycle
DIS-estrus	5	49
DIS-day 4	8	38
DIS-day 15	9	38
Control	9	41
	31	Mean 40.7 ± 1.8

Cycle Length Following DIS Infusion

Intrauterine infusion of 255 ml of DIS on day 4 of the cycle shortened that cycle in all cows to an average of 10.6 ± 0.1 days (P<0.01) while the same infusion on day 15 lengthened that cycle to 25.1 ± 0.6 days (P<0.01) (Table 4). Cycle lengths of cows infused during estrus did not differ from controls (P>0.05). The estrous cycle following the treated cycle was of normal duration in all groups (Table 4). These results agree with the previously discussed reports of Yamauchi *et al.* (1967) and Nakahara *et al.* (1971b). However, the

Table 4. Effect of DIS infusion on the length of the estrous sycle in cows

Treatment	No.	Cycle Length		Interval from DIS to estrus
		Infusion	Subsequent	
DIS-estrus	5	22.4 ± 0.1	20.6 ± 1.1	22.4 ± 0.1[c]
DIS-day 4	8	10.6 ± 0.2[a]	22.4 ± 1.1	6.6 ± 0.2[c]
DIS-day 15	9	25.1 ± 0.6[b]	22.9 ± 1.0	10.1 ± 0.6[c]
Control	9	21.3 ± 0.7	21.6 ± 1.0	---

[a]Significantly ($P<0.01$) shorter than control cycles.

[b]Significantly ($P<0.01$) longer than control cycles.

[c]I ≠ II ≠ III ($P<.05$).

shortening effect of DIS infusion on day 4 contradicts Grunert's (1971) report of no alteration in cycle length occurring when cows were infused with iodine solution before day 12.

The interval from DIS infusion to estrus was calculated to compare early versus late luteal phase responses (Table 4) and analyzed by the linear contrast procedure described by Snedecor and Cochrane (1967). The mean interval from DIS infusion on day 4 to estrus was 6.6 ± 0.2 days while the interval to estrus following infusion on day 15 was 10.1 ± 0.6 days ($P<0.05$). This difference may be a function of CL size or sensitivity to the luteolytic factor.

Endometrial Biopsies

Suitability of Histologic Sections

Approximately 25% of the 105 endometrial sections in this study contained no surface epithelium which is similar to the 12% and 18%

reported by Skjerven (1956) and Edwards (1971), respectively. The amount of surface epithelium present on the remaining sections was highly variable. Therefore, a portion of the sections collected were of little value since the surface epithelium was required for the sections to be acceptable. Numbers involved were inadequate to correlate the incidence of this loss of epithelium with specific stages of the estrous cycle; however, Skjerven (1956) reported that it appeared to be easier to lose surface epithelium on biopsies taken near the time of estrus rather than during the luteal phase of the cycle. Skjerven (1956) stated that rough treatment of tissue after removal could contribute to loss of otherwise intact epithelium. Due to the unsuitability of some sections, sections were pooled by group for examination rather than by individual animal.

Evaluation of Endometrial Biopsies

Initial estrus. Sections taken during the estrus prior to infusion (Figure 1) had pseudostratified columnar epithelium with good cellular integrity. Vacuolization of the basal portion of the cytoplasm of the surface epithelium and separation of the epithelium from the basement membrane were commonly observed at all stages. Neutrophils, lymphocytes, plasma cells and eosinophils were the more common types of inflammatory cells seen. These cells were individually scattered and found to some degree in nearly all sections. Clones of inflammatory cells were not observed. Various degrees of edema and congestion of the stratum compactum and stratum spongiosum were present in sections taken during estrus. The endometrial glands were frequently filled with debris which was probably an artifact caused by the collection process. Sections taken during the estrus prior to infusion were consistent with the

descriptions by Skjerven (1956) and DeBois (1961) of endometrial sections taken at estrus from reproductively normal cows.

Luteal phase prior to infusion. Sections taken on day 4 (Figure 2) or day 15 prior to infusion did not differ markedly from sections taken during the estrus prior to infusion. Occasional inflammatory cells, mainly neutrophils and plasma cells, were present but may have been less frequent than in sections taken during estrus, as reported by Skjerven (1956) and De Bois (1961). Edema and congestion of blood vessels were less evident on day 4 and day 15 than during estrus.

Twenty-four hours after infusion. The surface epithelium was either destroyed or severely distorted in all cases (Figures 3, 5 and 7). The remaining areas of epithelium had undergone metaplasia to a low cuboidal type, a marked change from the pseudostratified columnar epithelium present 24 hours earlier. Extensive vacuolization and edema were present in the stratum compactum just beneath the basement membrane in all 3 groups infused with DIS. The cells in this damaged band of superficial epithelium had, for the most part, ruptured. Capillaries in the adjacent areas were engorged and prominent. Neutrophils were present in sections 24 hours after infusion at the approximate level expected during estrus in reproductively normal cows. These changes were observed in all treated groups 24 hours after DIS infusion (Figures 3, 5 and 7), although they appeared more pronounced following infusion on day 4 or day 15 than during estrus. The biopsy schedule in this study was not designed to detect the maximum neutrophilic response as Grunert (1971) found the leukocytic response to persist for 3 to 5 days after infusion. The endometrial glands and deeper areas of the stratum spongiosum were not affected. These histopathologic changes were

similar to those described by Grunert (1971) for the same situation.

The described endometrial reaction to DIS would appear to be sufficient to release or destroy a luteolytic factor contained in the cells of that area. It would appear that the differences in the effect of DIS infusion on cycle length (Table 4) were not due to differences in the endometrial response to the infusion.

Estrus following infusion. The surface epithelium had regenerated by the estrus following infusion in all groups (Figures 4, 6 and 8), even though the interval from infusion to estrus (Table 4) varied from 7 days for Group II to 22.4 days for Group I. This would be consistent with Grunert's (1971) report that uterine epithelium was to a great extent regenerated by the 5th day following DIS infusion. Leukocytic infiltration was present in the stratum compactum similar to that seen during estrus prior to infusion. Congestion and edema were also found in the stratum compactum at this time. Where the interval from infusion to estrus was short (Groups II and III) the stratum compactum appeared to be less compact and more spongy at the estrus following infusion than at the previous estrus. This may indicate incomplete repair of this layer at this stage.

Serum Progesterone and LH Following DIS

Neither serum progesterone nor LH concentrations for control cows and cows infused during estrus differed ($P>0.05$) during the treated cycle when statistically analyzed by the split plot method of Gill and Hafs (1971). Likewise progesterone and LH concentrations for cows infused on day 15 did not differ from these two groups from day 4 up to the time of infusion on day 15. Similarly, progesterone and LH concentrations were statistically similar for all groups from estrus through day 4.

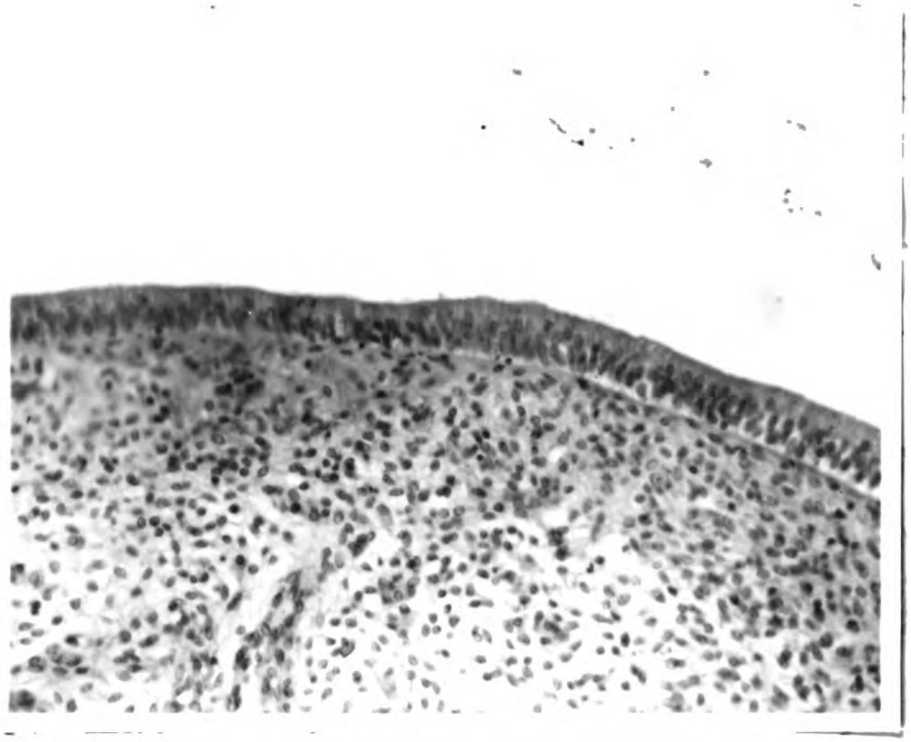

Figure 1. Bovine endometrium at estrus. Pseudostratified columnar epithelium is present. Group II cow (No. 1035) (x 200).

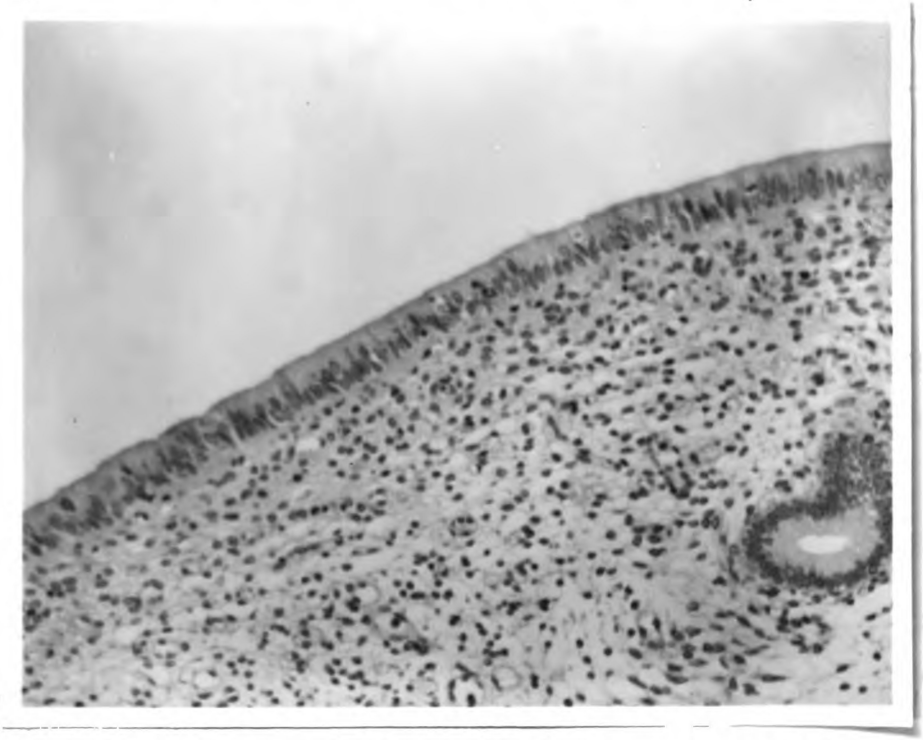

Figure 2. Bovine endometrium at day 4. Pseudostratified columnar epithelium is present. Stratum compactum is normal with some neutrophils present. Group II cow (No. 976) (x 200).

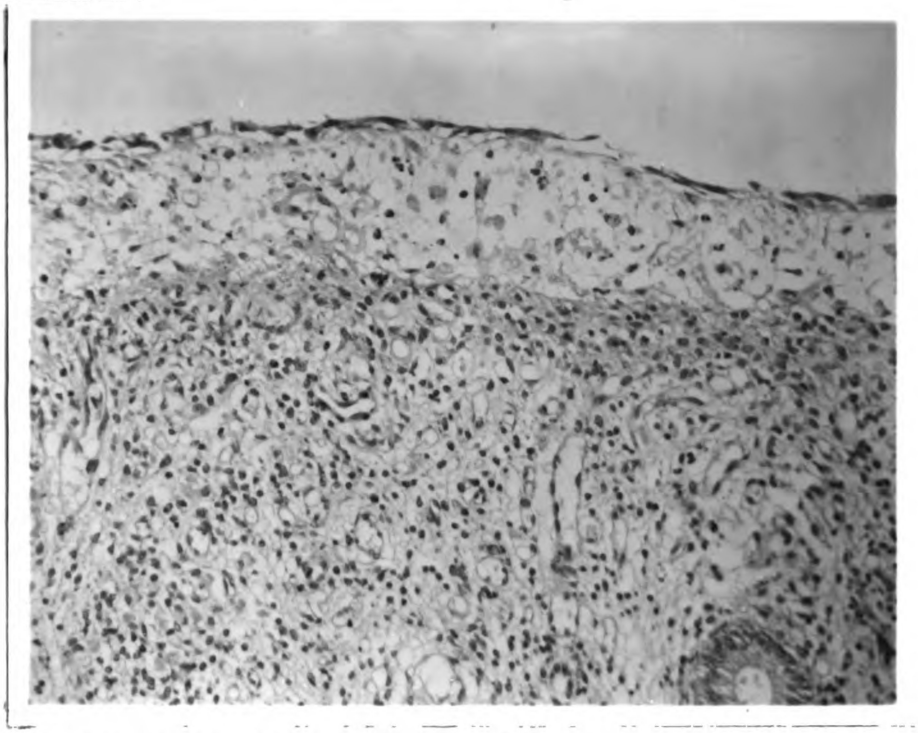

Figure 3. Bovine endometrium at day 5, 24 hours after infusion of DIS. Surface epithelium is compressed to low cuboidal form. Edema, vacuolization and cellular destruction appear in the stratum compactum. Group II cow (No. 1106) (x 200).

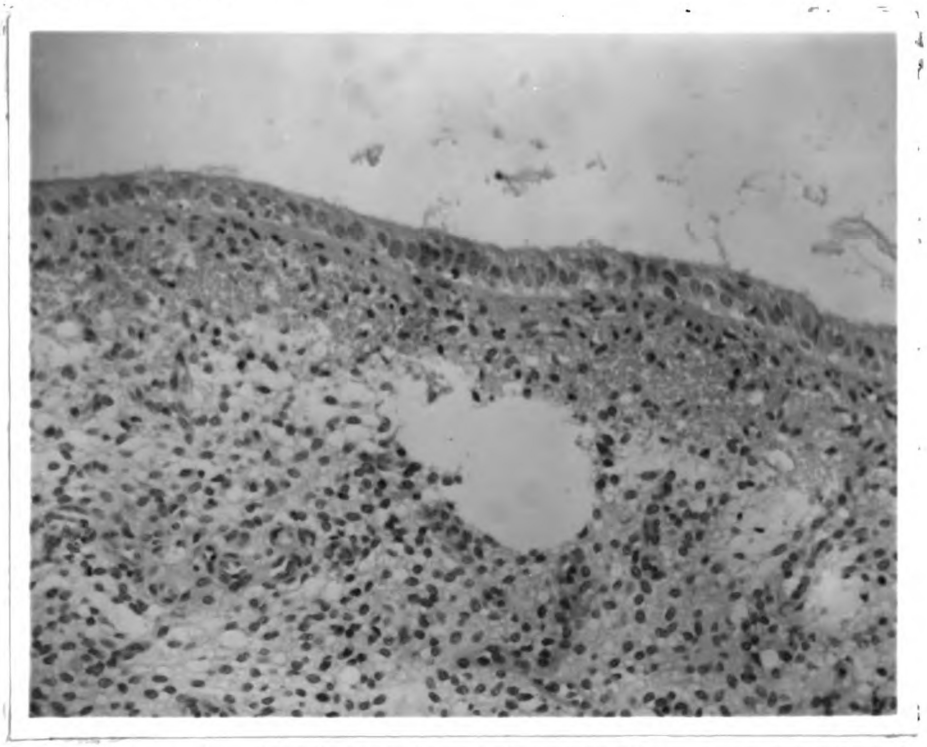

Figure 4. Bovine endometrium at the estrus following infusion of DIS on day 4. Pseudostratified columnar epithelium has regenerated. Some edema and vacuolization are present in the stratum compactum. Group II cow (No. 1035) (x 200).

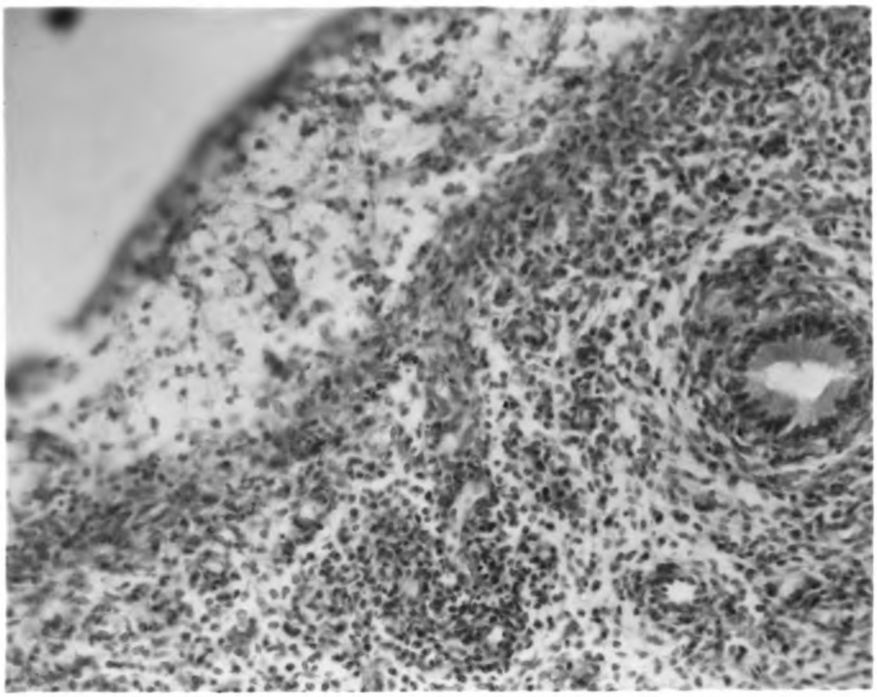

Figure 5. Bovine endometrium at day 16, 24 hours after infusion of DIS. Surface epithelium has sloughed. Superficial parts of stratum compactum are extensively disrupted. Neutrophils are present in the deeper areas of stratum compactum. Group III cow (No. 1100) (x 200).

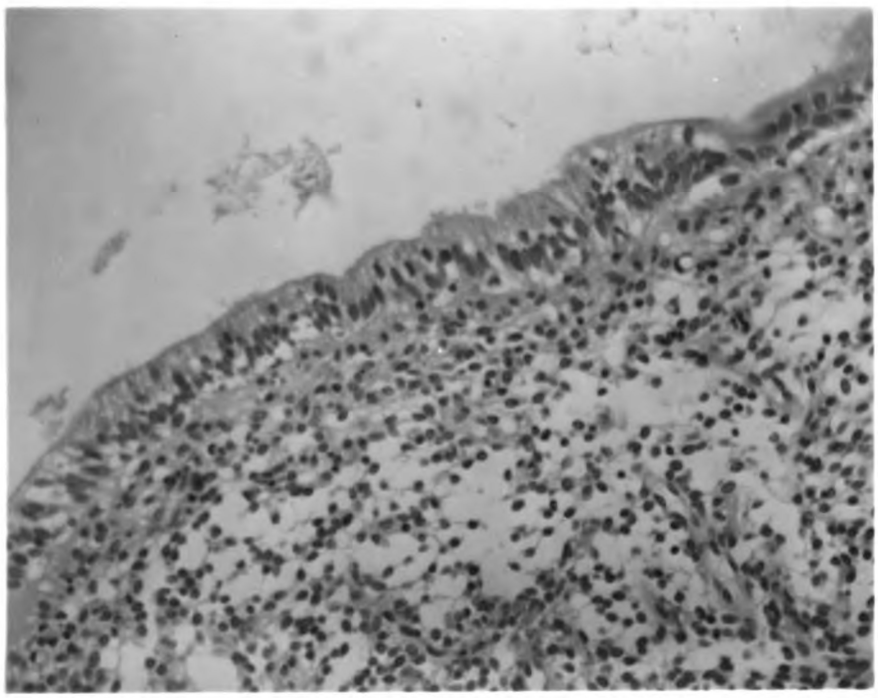

Figure 6. Bovine endometrium at the estrus following infusion of DIS on day 15. Pseudostratified columnar epithelium has regenerated. Edema is evident in stratum compactum. Group III cow (No. 1122) (x 200).

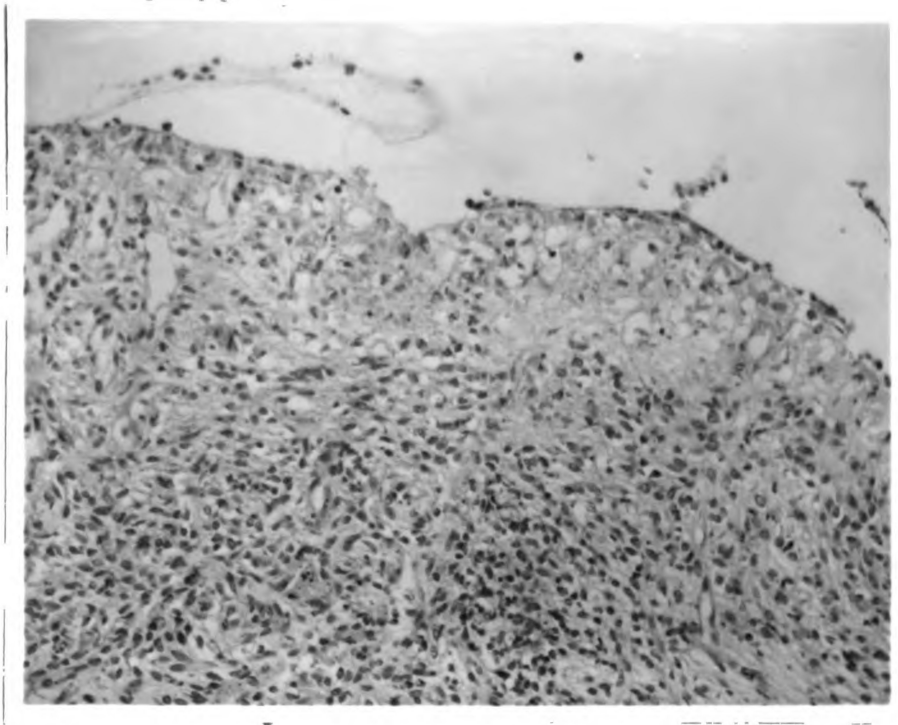

Figure 7. Bovine endometrium at day 1, 24 hours after infusion of DIS. Surface epithelium is almost completely destroyed. Vacuolization and cellular destruction are present in stratum compactum. Group I cow (No. 1084) (x 200).

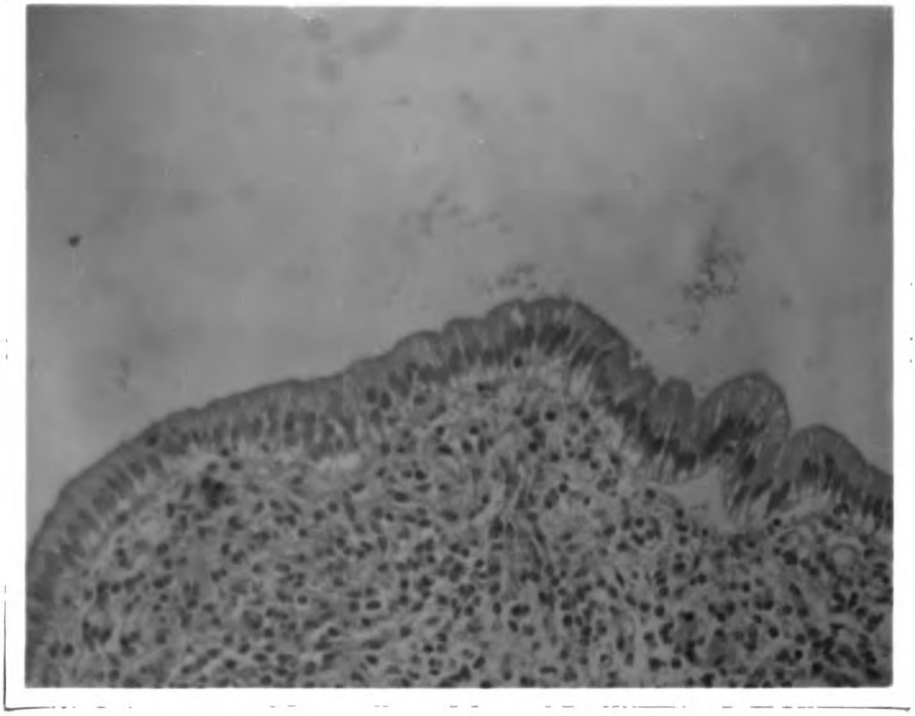

Figure 8. Bovine endometrium at the estrus following infusion of DIS on day 0. Pseudostratified columnar epithelium is present. Stratum compactum is normal. Group I cow (No. 937) (x 200).

The serum progesterone in control cows and cows infused during estrus (Figure 10) followed the same pattern as that described for the normal bovine estrous cycle by Shemesh *et al.* (1968), Pope *et al.* (1969), Stabenfeldt *et al.* (1969), Hansel and Snook (1970), Henricks *et al.* (1970), Lamond *et al.* (1971), Swanson *et al.* (1972) and Wettemann *et al.* (1972). Following DIS infusion on day 4, progesterone levels increased from day 4 to day 7 as in normal estrous cycles. It would appear (Figure 10) that infusion of DIS on day 4 stimulated increased progesterone synthesis and release through day 7; however, this difference was not significant ($P>0.05$) when time trends as described by Gill and Hafs (1971) were considered. Further research in this area may indicate that early luteal regression after infusion of DIS on day 4 is due to excessive stimulation and early "burn-out" of luteal tissue. Progesterone then fell sharply over the 3-day period from day 7 through day 10 back to baseline levels ($<$ 1.0 ng/ml). This period of sharply declining progesterone was very similar to the progesterone decline in Groups I and IV between days 17 and 20. Development of the Cl through day 6 or 7 and rapid CL regression on days 7 and 8 were detected by palpation *per rectum* in Group II.

Following infusion of DIS on day 15, serum progesterone remained at luteal phase levels until about day 21 (Figure 10) or approximately 6 days longer than Groups I and IV. The fluctuation in serum progesterone (Figure 10) from day 15 to 21 following infusion of DIS on day 15 probably reflects normal variation in the assay and in serum levels of progesterone. However, further work may indicate that this is the actual response following infusion of DIS on day 15. The rapid decline in serum progesterone from day 21 to day 24 was similar to the progesterone decline noted at other intervals in other groups. Marked regression of the CL, as

Figure 9. Serum LH concentrations during estrous cycles in cows infused with DIS at estrus, day 4 or day 15.

Figure 10. Serum progesterone concentrations during estrous cycles in cows infused with DIS at estrus, day 4 or day 15.

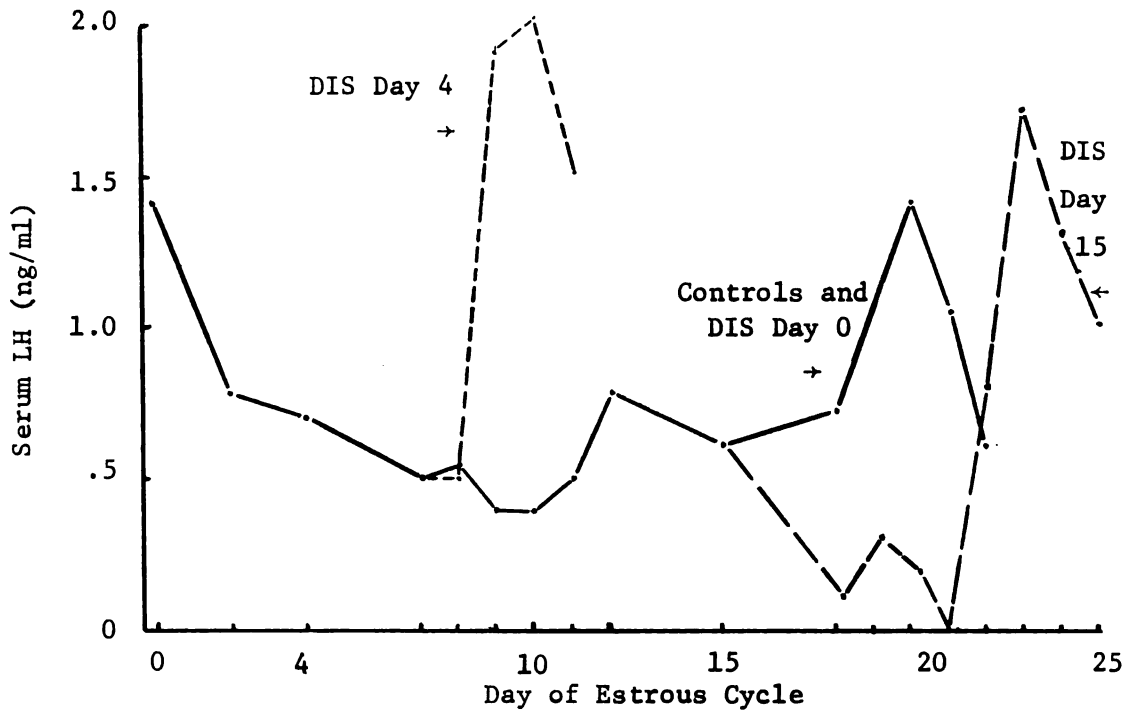

Figure 9

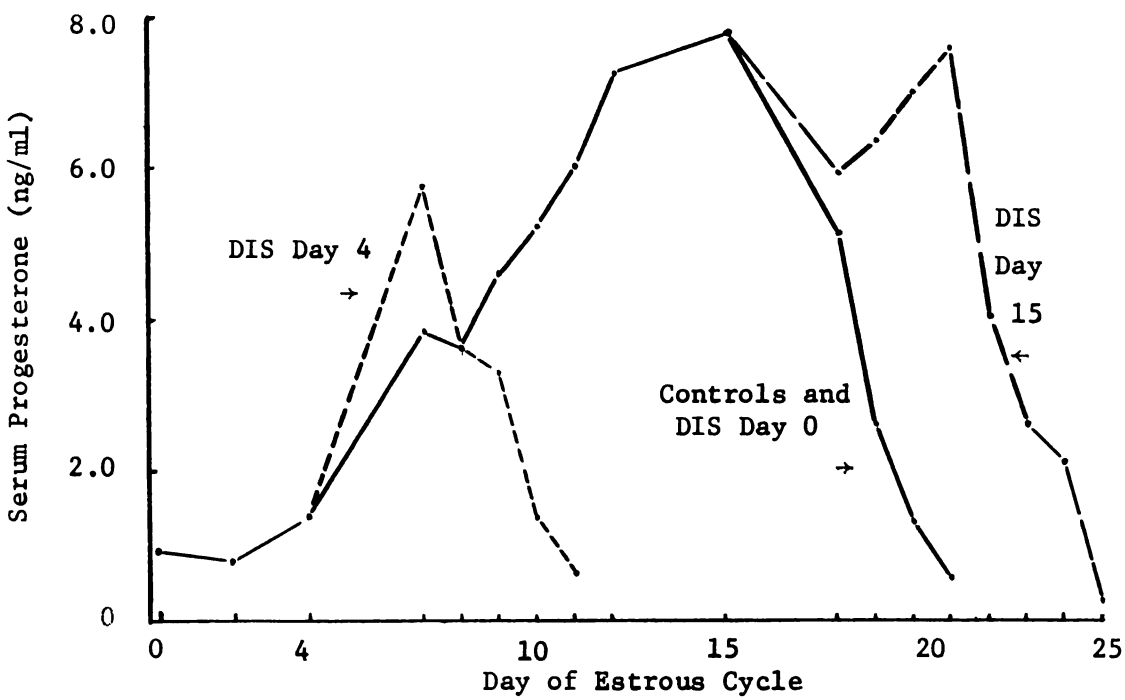

Figure 10

detected by palpation *per rectum*, was delayed until day 21 to 23 of the cycle.

Serum LH in control cows and cows infused with DIS during estrus (Figure 9) followed the same pattern as reported for normal bovine estrous cycles by Hansel and Snook (1970), Henricks *et al.* (1970), Swanson and Hafs (1971), Swanson *et al.* (1972) and Wettemann *et al.* (1972). The low LH peaks in Figure 9 reflect an averaging effect. Since cows were bled only once daily during estrus and peak levels of LH last only 6 to 10 hours, many LH peaks probably were missed. After infusion of DIS on day 4, LH peaked on days 9 and 10. The infusion of DIS on day 15 of the cycle delayed the LH peak until day 23. In both cases the LH peak occurred as serum progesterone was reaching baseline levels so the normal relationship between the progesterone decline and the LH peak was maintained even though length of the cycles had been altered.

Infusion of DIS on day 4 or day 15 altered the length of the estrous cycle by altering the length of the luteal phase of the cycle. The proestrous period of the cycle was not altered in these groups. The normal luteolytic sequence appears to be functioning but DIS infusion has altered the time of onset of the luteolytic process.

Subsequent Reproductive Performance

The mean postpartum interval to first service and the mean services per conception (Table 5) did not differ among the four groups ($P>0.05$). However, the mean days open was less ($P<0.01$) for the controls than for the three groups which received DIS (Table 5). Since the interval to first service and services per conception were not different, a high incidence of long irregular cycles in the groups receiving DIS might be anticipated. Five cows in Groups II and III had cycles ranging from 40

Table 5. Reproductive performance following infusion of DIS

Treatment	No.	Interval to first service	Days open	Services/ conception*	Open cows (%)
DIS-estrus	5	79	108	2.4	0
DIS-day 4	8	67	122	2.4	13
DIS-day 15	9	70	124	2.8	11
Control	9	64	81[a]	1.9	22[b]
Mean		68.8	109	2.4	90

[a]Significantly ($P<0.01$) lower than the group means for cows which received DIS.

[b]One cow died of malignant lymphoma before conceiving.

*Open cows were not considered in services per conception or in days open.

to 62 days after being bred at least once. The causes of these long cycles were not determined. Long irregular cycles can be caused by several factors including missed or silent heats, early embryonic mortality associated with conditions such as endometritis, and cystic ovarian follicles. The physiological relationship between the long cycles observed in these cows in Groups II and III and the experimental procedure is not known. Cows in these groups were infused during the luteal phase of the estrous cycle and Black *et al.* (1953) reported that the bovine uterus was more susceptible to infection during the luteal phase of the estrous cycle than during the follicular phase. The significant difference in days open between Groups I and IV was apparently due to those two groups being the extremes in the range of means for the interval to first service (Table 5).

Four cows did not conceive following this experiment and were not included in services per conception or days open (Table 5). One cow (No. 888) in Group IV died of malignant lymphoma before conceiving. Three cows (Nos. 880, 937 and 1088 of Groups II, III and IV) were sold open after 7, 10 and 8 services, respectively.

Effect of $PGF_{2\alpha}$ Following DIS

The luteolytic effect of $PGF_{2\alpha}$ reported by Louis *et al.* (1972) was evident after the endometrium had been damaged by intrauterine infusion of DIS (Figures 11 and 12). Plasma progesterone levels dropped sharply (Figure 12) in the first 24 hours after $PGF_{2\alpha}$ was administered. The reason for the difference in progesterone concentrations at day 15 (Figure 12) is not known but, as described in the Materials and Methods section of this thesis, different assay methods were used for each group. The size of the CL as determined by palpation *per rectum* was reduced in the first 24 to 48 hours after infusion of $PGF_{2\alpha}$. The LH surge (Figure 11) and behavioral estrus with ovulation followed in the normal manner. Intrauterine infusion of 5 mg of $PGF_{2\alpha}$ 24 hours after infusion of DIS on day 15 shortened ($P<0.01$) the estrous cycle when compared to cows receiving only DIS (Table 6). From these results it would appear that the endometrial damage caused by DIS infusion, as described earlier, did not alter the luteolytic activity of $PGF_{2\alpha}$ administered intrauterinally.

General Discussion

The alteration in the bovine estrous cycle length following intrauterine infusion of DIS in the early and the late luteal phases of the estrous cycle observed in this experiment agrees with the previously discussed reports by Morrow *et al.* (1971) and Nakahara *et al.* (1971b)

Figure 11. Serum LH in cows following infusion of DIS on day 15 versus DIS on day 15 plus $PGF_{2\alpha}$ on day 16.

Figure 12. Serum progesterone in cows following infusion of DIS on day 15 versus DIS on day 15 plus $PGF_{2\alpha}$ on day 16.

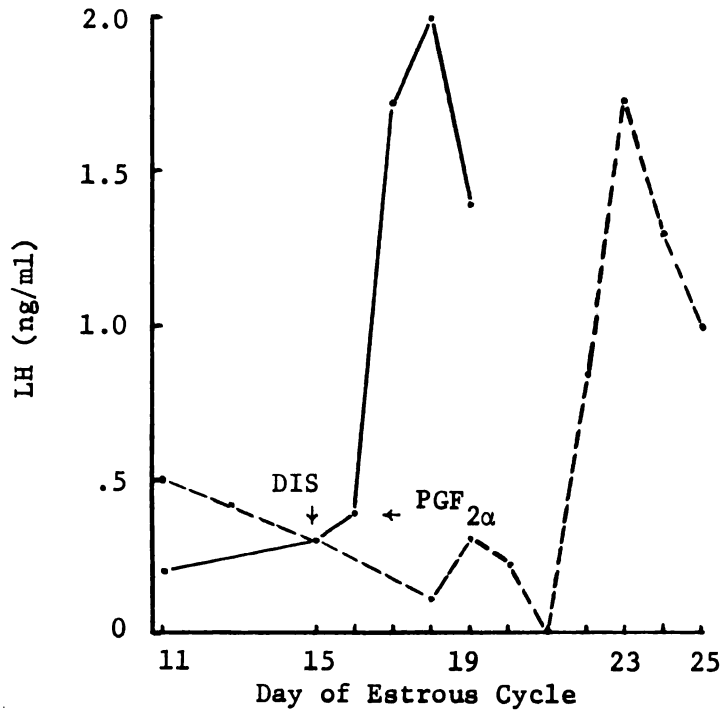

Figure 11

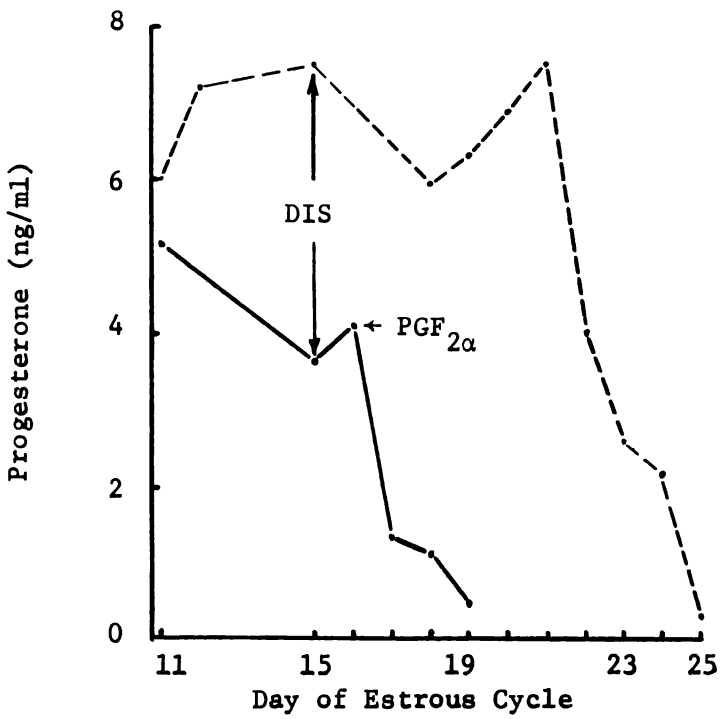

Figure 12

Table 6. Effect of $PGF_{2\alpha}$ after infusion of DIS on cycle length in cows

Treatment	No.	Cycle Length
DIS-day 15	9	25.1 ± 0.6
DIS-day 15 + $PGF_{2\alpha}$-day 16	5	19.1 ± 0.2[a]

[a]Significantly ($P<0.01$) shorter than cycle lengths in cows which received only DIS.

The endometrial response observed in this experiment 24 hours after infusion of DIS at estrus, day 4 or day 15 did not differ. Therefore the variation in the intervals from infusion of DIS to estrus observed for the various groups cannot be explained by different endometrial responses to DIS.

From the LH and progesterone concentrations it appears that infusion of DIS on day 4 or day 15 acts by altering the time of onset of the normal luteolytic process but does not alter the sequence of events of the luteolytic process. The progesterone decline and LH peak found in each group during a three-day period prior to estrus were typical of the proestrous period in normal estrous cycles.

The literature reviewed on utero-ovarian interaction indicated that the endometrial irritation caused by DIS infusion is probably altering cycle length by affecting the uterine luteolytic factors. Release of the luteolytic factor or destruction of the source of the luteolytic factor following endometrial irritation are possibilities in view of the endometrial damage present 24 hours after DIS infusion. From reports by Louis *et al.* (1972) and Rowson *et al.* (1972) we know that estrus occurs about 3 days after intrauterine infusion of $PGF_{2\alpha}$.

Assuming that $PGF_{2\alpha}$ is the uterine luteolytic factor, estrus should occur 3 to 4 days after endometrial irritation if DIS infusion caused release of the luteolytic factor. The 7- and 10-day intervals from infusion to estrus following infusion on day 4 and day 15, respectively, seem to indicate that DIS infusion is destroying or removing the source of the luteolytic factor rather than releasing it. The 7- and 10-day intervals may be sufficient to allow endometrial repair and renewed synthesis of the luteolytic factor. Grunert *et al.* (1971) reported that the uterine epithelium and superficial endometrium can regenerate in 4 to 5 days following infusion of iodine solution. So the combined time interval for endometrial repair and $PGF_{2\alpha}$ luteolytic action would approximate the 7- and 10-day intervals observed from DIS infusion to estrus. If this hypothesis is correct, the stimulus for release of the luteolytic factor following endometrial repair is not known. The significant variation ($P<0.05$) in the intervals from DIS infusion to estrus (7 versus 10 days) may be a function of CL size and the production rate of the luteolytic factor. Another possibility may be variation in sensitivity of the CL to the luteolytic factor during the estrous cycle. The absence of active luteal tissue at estrus probably explains the failure of DIS infusion to alter the cycle length at that time.

The results of this experiment indicate that the same mechanism of action may be involved even though DIS infusion on day 4 shortened the estrous cycle while DIS infusion on day 15 lengthened the estrous cycle. When the interval from infusion to estrus and the size of the CL at the time of the respective infusions are considered it appears that the same processes may be occurring in both cases. The shortening effect of infusion on day 4 and the lengthening effect of infusion on day 15

may be functions of the time of DIS infusions rather than reflections of two different responses to intrauterine infusion of DIS.

Yamauchi *et al.* (1967), Morrow *et al.* (1971) and Nakahara *et al.* (1971b) reported that infusion of iodine solution during the mid-luteal phase had no effect on cycle length. Nakahara *et al.* (1971b) found that the interval from infusion of iodine solution to estrus at day 9 or day 13 was 10 days. Similar infusion on day 10, 11 or 12 with a 10-day interval from infusion to estrus would produce a cycle of normal duration and seem to be ineffective even though the same sequence of events as described in this experiment were occurring.

Rajakowski (1960) described two waves of follicular growth during the estrous cycle in the cow. The first started at day 3 or 4 and the second started between days 12 and 14. Initially, in both cases, several follicles developed to exceed 5 mm in diameter and then one large follicle emerged while the remainder regressed. The large follicle of the first wave developed through day 11 and then underwent cystic atresia. In the second wave one large follicle also developed while the remainder regressed and this large follicle became the ovulatory follicle at the subsequent estrus. Following infusion of DIS on day 4 the large follicle of the first wave of growth apparently became the ovulatory follicle. Whether the interval from infusion at day 4 to estrus was dependent upon the development of this large follicle or was merely coincidental with its development is not known. These waves of developing follicles may be a key factor in regulating the onset of estrus following luteolysis.

Uterine distention has been eliminated by Nakahara *et al.* (1971a) as the responsible mechanism for cycle alteration following iodine solution infusion. Small volumes of iodine solution, too small to cause

uterine distention, were able to alter the length of the estrous cycle. Morrow *et al.* (1971) reported that intrauterine infusion of 250 ml of physiologic saline on day 1, day 4 or day 11 did not alter cycle lengths but a similar volume of dilute iodine solution did shorten the cycle when used on day 4. These reports indicate that uterine irritation, and not uterine distention, is the mechanism responsible for cycle alteration following infusion of DIS.

In the second experiment the luteolytic activity of $PGF_{2\alpha}$ was evident 24 hours after the uterine endometrium had been damaged by DIS infusion. The cycle lengthening effect of DIS infusion on day 15 was overcome by administration of $PGF_{2\alpha}$ on day 16 with estrus occurring on day 19. These data support the previously discussed hypothesis that DIS infusion destroys or removes the source of the uterine luteolytic factor rather than releasing it. If DIS released the luteolytic factor, estrus should have occurred about 3 days later, as observed here following DIS plus $PGF_{2\alpha}$ infusion rather than 10 days later as observed when only DIS was infused.

The fact that the endometrial damage caused by intrauterine infusion of DIS did not block the luteolytic activity of $PGF_{2\alpha}$ adds support to the hypothesis that $PGF_{2\alpha}$ is the uterine luteolytic factor in the cow. This indicates that the superficial endometrium is not needed for the luteolytic action of $PGF_{2\alpha}$. In other words, $PGF_{2\alpha}$ may be acting directly on the ovarian luteal tissue rather than activating or stimulating some component of the superficial endometrium. Prostaglandin $F_{2\alpha}$ needs to be tested in hysterectomized animals to determine if the uterus is necessary for the luteolytic activity of $PGF_{2\alpha}$. Lukaszewaska, Wilson and Hansel (1972) have demonstrated that $PGF_{2\alpha}$ is luteolytic in pseudopregnant hysterectomized hamsters. The possibility of $PGF_{2\alpha}$

exerting its luteolytic effect via an indirect route through some organ or tissue other than the uterus has not been eliminated. These results are consistent with the proposal of $PGF_{2\alpha}$ being a uterine luteolytic factor in the cow.

SUMMARY

The effect of intrauterine infusion of dilute iodine solution (DIS) on serum progesterone and LH levels, estrous cycle length and the endometrium of the uterus was studied in 31 postpartum dairy cows.

Infusion of DIS at estrus, day 4 or day 15 caused similar damage to the epithelium and superficial endometrium of the uterus. Surface epithelium was destroyed or severely distorted in all cases. Edema and vacuolization of the superficial endometrium with destruction of normal cellular detail occurred in all three groups. However, infusion during estrus did not alter cycle length or serum progesterone or LH levels during the following estrous cycle. Infusion of DIS on day 4 significantly ($P<0.01$) shortened the estrous cycle and hastened progesterone decline and the LH peak. Infusion of DIS on day 15 delayed ($P<0.01$) the onset of estrus, progesterone decline and the LH peak. The alterations in cycle length were due to altered length of the luteal phase with no changes observed in the proestrous phase of the cycle. Even though the intervals from DIS infusion to estrus following day 4 or day 15 infusion were different ($P<0.05$) the same sequence of events may be occurring in both cases. The interval difference may reflect the difference in response between a growing CL and a mature CL to the luteolytic factor. The 7- and 10-day intervals observed in these groups indicate that infusion of DIS destroyed the luteolytic factor rather than releasing it in active form. Subsequent reproductive performance may have been adversely affected by this experimental procedure.

In a second experiment the effect of intrauterine infusion of $PGF_{2\alpha}$, a luteolytic substance, was studied after the endometrium had been damaged by infusion of DIS. The endometrial damage did not alter the luteolytic action of $PGF_{2\alpha}$ indicating that $PGF_{2\alpha}$ probably acts directly on the ovarian luteal tissue. The shortened interval from infusion of $PGF_{2\alpha}$ to estrus after the endometrium had been damaged by infusion of DIS is additional evidence that DIS destroys the luteolytic factor rather than releasing it in active form. These results are consistent with, but not proof of, the proposal of $PGF_{2\alpha}$ being the uterine luteolytic factor in the cow.

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BIOGRAPHICAL SKETCH

BIOGRAPHICAL SKETCH

Bradley Edson Seguin^{II} was born on December 22, 1944, in Black River Falls, Wisconsin. He attended public schools in Alma Center, Wisconsin, and graduated from high school in June 1963. Following three semesters in the civil engineering curriculum at the University of Wisconsin, he decided to follow his interest in animal agriculture and completed the preveterinary medicine requirements at Wisconsin State University - River Falls in June 1966. He was accepted into the University of Minnesota's College of Veterinary Medicine and received the Bachelor of Science degree in June 1968 and the Doctor of Veterinary Medicine degree in June 1970.

Pursuing his interest in theriogenology, he served a one-year internship at the Animal Reproduction Laboratory at Colorado State University, Fort Collins. He was granted a Post-DVM Fellowship in September 1971 at Michigan State University in the Department of Large Animal Surgery and Medicine and received the Master of Science degree in December 1972.