EFFECT OF EXOGENOUS GONADOTROPIN RELEASING HORMONE ON RECOVERY FROM SEPTIC UTERINE CONDITIONS IN POSTPARTUM DAIRY COWS

by

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(ABSTRACT)

Lactating Holstein cows were assigned to clinical groups (CG; retained placenta, RP, n=20; uterine infection, UI, n=22; control, C, n=18) and given GnRH (200ug,im) or saline (T) on d 15 postpartum, to evaluate reproductive tract involution, uterine discharge, bacterial population and inflammation, ovarian activity, reproductive efficiency and plasma progesterone, glucose, and urea. Reproductive tract involution was not affected by CG or T and was complete by 40 d postpartum (DPP). Purulent vaginal discharge was found in UI, but improved over DPP. C , UI and RP had plasma progesterone concentrations > 1 ng/ml at 21, 27 and 29 DPP, respectively. Prolonged luteal phases resulted when UI was treated with GnRH. RP plasma urea was 20, 16.5 and 21.5 mg/100ml on 15, 35 and 50 DPP. UI had 16.3, 14.7 and 16.8 mg/100 ml on 15, 35 and 50 DPP. C and had 12.2, 14.3 and 12.8 mg urea/100ml on 15, 30 and 50 DPP. Plasma glucose ranged from 63 to 61.2 mg/100 ml between d 15 and 50. C. pyogenes and E. coli incidences were 53 and 22, 35 and 37, and 12 and 41% for RP, UI and C, respectively. Neutrophils were found in 58% of GnRH treated cows. Diffuse lymphocytes occurred in RP, 54%; UI, 40.5% and in C, only 5.4%. Days open were 134, 117 and 97 and services per conception were 2.5, 1.7 and 1.6 for RP, UI and C, respectively. RP and UI had increased time to plasma progesterone elevation, increased plasma urea concentration and increased days open. GnRH did not benificially affect reproductive efficiency.

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CHAPTER I

INTRODUCTION

A prolonged interval between calving and conception results in economic losses to the dairy farmer through decreased milk production. Complications during calving often lead to septic conditions of the reproductive tract. Poor uterine health results in further monetary losses to the dairy farmer by causing increased veterinary bills and increased breeding costs due to lowered fertility.

At calving the uterus is considered a sterile environment. However, following calving the cervix remains open for several days allowing the invasion of pathogenic organisms. Animals which require manual assistance during calving are especially prone to infection. Additionally, cows which retain the fetal membranes have an increased infection rate as the membranes act as a wick to draw bacteria into the uterus. Cellular debris remaining in the uterus from the conceptus provides an ideal environment for the growth of these pathogens. In some cows the combination of uterine infection and postpartum stress leads to an alteration in lifespan of the ovarian corpus luteum (CL). Animals which ovulate within 15 d of parturition seem especially prone to this condition. By prolonging CL lifespan the number of possible ovulations prior to the desired time of insemination are decreased.

Nutrition has been shown to play a role in reproduction. Elevated plasma urea concentrations produce a uterine environment which is not conducive to gamete survival. Depressed plasma glucose at the time of insemination also has been related to decreased fertility.

Several hormonal regimens have been proposed to increase fertility in the postpartum cow. Among these is the injection of gonadotropin releasing hormone (GnRH) on approximately d 15 postpartum. Injection of this hormone has been shown to cause ovulation in the early postpartum cow. With each ovulation postpartum, the cow's fertility increases. Therefore, GnRH increases the number of ovulations prior to breeding and thereby improves conception rate to the first insemination postpartum.

The effect of GnRH on cows with clinical reproductive conditions in the early postpartum period has not been previously studied. This study was undertaken to evaluate the effect of GnRH injection in cows with uterine infections or retained fetal membranes and to determine endocrine and blood metabolite changes during the early postpartum period and subsequent fertility.

CHAPTER II

LITERATURE REVIEW

The prompt return to cyclical ovarian activity following parturition in dairy cattle is essential to maintain maximum productivity (Lamming and Bulman, 1976). Through increased breeding costs, medications and calf and milk losses, infertility costs dairymen over $116 per cow per year (Pelissier, 1982). A cow's fertility increases with each ovulation postpartum (Bosu, 1982). Therefore, it is essential that dairy cattle return to a normal endocrine state as soon as possible following calving. This allows for more ovulations prior to the first postpartum service and increased conception rate.

Endocrine Relationships

A number of factors may cause a prolonged postpartum anestrous period, including retained placental membranes, septic metritis, delayed involution of the reproductive tract, prolonged luteal phase, cystic ovaries, and high milk yield (Fonseca et al., 1983; Bosu, 1982; Oltenacu et al., 1983).

It appears that the first ovulation postpartum may be delayed due to the suppression of gonadotropin releasing hormone (GnRH) activity on the pituitary. However, pituitary binding capability does not change with time in the postpartum beef cow. The number and affinity constant of binding sites in acyclic cows remain the same from d 7 to 42 postpartum. However, the ability of the pituitary to release LH into the plasma in response to exogenous GnRH doubled from 17.1 ng/ml to 34.8 ng/ml over this same time period. Therefore, it appears that mechanisms other than occupation of GnRH receptor sites must inhibit a preovulatory luteinizing hormone (LH) profile in the early postpartum period (Leung et al., 1986).

Work with beef cows has shown that early postpartum follicles have a steriod content and gonadotropin receptor profile similar to that of cycling cows. Therefore, lack of follicular activity in the early postpartum cow is not due to lack of ovarian competence (Braden et al., 1986).

Kesler et al. (1977) injected dairy cows with GnRH on days 1 or 2, 3 or 4, 5 or 6, 7 or 8, 12 or 13, and 18 or 19 postpartum. It was found that prior to day 6 postpartum the pituitary was not responsive to GnRH and there was no subsequent rise in plasma LH concentrations. After d 7 postpartum plasma LH concentrations increased within .5 h following GnRH injection and returned to pre-injection values by 6 h post GnRH injection.

Estradiol-17β and LH concentrations prior to GnRH injection appear important in mediating a response to exogenous GnRH. Release of LH in response to estrogen implants was negatively correlated to day postpartum in beef cows (Peters and Lamming, 1984). Because plasma estrogen concentrations increase without a concurrent increase in LH concentration, it appears that estrogen sensitivity at the pituitary level is decreased in the early postpartum cow. As pituitary sensitivity is indicated in illiciting preovulatory gonadotropin surges in several species (Karsch et al., 1980), it is possible a similar mechanism must be initiated to induce ovulation in the postpartum cow.

Positive feedback of estradiol-17β on the pituitary from developing ovarian follicles is most likely responsible for increased plasma LH concentration with increased time postpartum (Kesler et al., 1977). Peters et al. (1985) were able to induce LH peaks between d 3 and 6 postpartum by injecting 2.5 ug GnRH every 2 h for 48 h, although LH peaks were higher in cows similarly treated between d 7 and 8 postpartum. All cows showed increased estradiol 17β concentrations indicative of ovarian response to gonadotropins. However only 1 of 9 cows exhibited a preovulatory type increase in estradiol-17β, LH and follicle stimulating hormone (FSH). This further suggests that pituitary insensitivity to estradiol feedback prevents preovulatory LH/FSH surges in the early postpartum cow.

Plasma progesterone concentrations prior to GnRH injection do not effect subsequent LH release. However, Rutter and coworkers (1985) compared d 7 CL formation from cows which had received progesterone for 4 d prior to GnRH injection to CL from cows which had received sham oil injections prior to GnRH. The progesterone treated cows had significantly fewer short luteal phases in response to induced ovulation than the non-treated cows, suggesting that preovulatory progesterone concentration was involved with subsequent luteal function.

Shortened cycles and low progesterone concentrations found in early postpartum cows may be the result of insufficient plasma LH to bring about proper luteinization of a follicle. Several researchers have indicated that both the magnitude and frequency of pulsatile LH release must exceed a threshold to cause the preovulatory surge necessary for ovulation. Goodale et al.(1978) reported increases in the magnitude and frequency of LH release from d 2 to 3 postpartum to d 12 to 13 postpartum. On d 2 to 3 postpartum no cows showed hourly changes in plasma LH concentrations of more than 1 ng/ml, while on d 12 to 13 postpartum 6 of 10 cows had peaks of this magnitude. Additionally, the number of animals displaying more than two LH pulses in a 10 h period increased from one on d 2 to 3 postpartum to six on d 12 to 13 postpartum. However, the increase in LH pulsatile activity was not related to ovarian structures or interval to first ovulation. Others (Riley et al., 1981) have found the converse to be true, that is, pulsatile LH release is correlated to the first postpartum ovulation.

Use of GnRH in Early Postpartum Cows

Gonadotropin releasing hormone has been indicated for the treatment of ovarian abnormalities and uterine infections following the retention of placental membranes (Bosu, 1982; Bostedt et al., 1980). Britt et al.(1974) reported that implanting cows with GnRH on d 14 postpartum caused a (LH) surge and led to ovulation the following day. Similar results were reported by Cavestany and Foote (1985) following injection of a GnRH analog.

Britt et al. (1974) found that involution of the previously gravid horn occurred more quickly in cows which received GnRH than in those which did not. This agrees with the findings of Bostedt et al. (1980) in which uterine and cervical involution rates were increased by GnRH injections on d 10 or 12 postpartum. However, Oltenacu et al. (1983) found that 50, 100 or 200 ug of GnRH administered intramuscularly between 8 and 21 d postpartum had no effect reproductive tract involution, days open or services per conception.

Etherington et al.(1983) studied the effect of GnRH (250 ug, im) injection on d 15 in 152 dairy cows. Gonadotropin releasing hormone treatment caused a 26 d increase in days to first heat following calving (60 d in control cows vs. 86 d for animals receiving GnRH). Additionally, days to first service were increased by 24 and days open increased by 15. Services per conception and number of observed heats prior to breeding were not affected by GnRH treatment. These researchers attributed the reduced reproductive efficiency observed to pyometra brought about by GnRH administration.

Involution of the Reproductive Tract

Involution rate of the cervix is slowed by abnormalities at calving or in the early postpartum period (Fonseca, 1983). The presence of an abnormal uterine discharge also slows involution.

Prostaglandin release by the uterus has been implicated in the involution process (Lindell et al., 1982). Elevated plasma prostaglandins increase the involution rate , however, ovulation does not occur until prostaglandins have returned to prepartum values. Therefore, delayed periods of marginally elevated prostaglandins both decrease involution rate and increase days to first service.

Delayed cervical involution leads to increased time to first estrus (5d) and increased days open by 11 to 22 d (Oltenacu et al., 1983; Fonseca et al., 1983). However, in the evaluation of 1738 Holstein lactation records, Dohoo (1983) found no difference in services per conception, conception rate at first service or days open in normal cows, cows with uterine infection at d 60 postpartum or cows with a health problem at calving such as retained placenta, ketosis, milk fever or dystocia.

Bacteriology of the Uterus

Peak preovulatory plasma LH concentrations increase with each ovulation postpartum. The first postpartum cycles are often shortened with low plasma concentrations of progesterone. The second cycle can be lengthened by the presence of uterine endometritis or delayed uterine involution (Oltenacu et al., 1983; Fonseca et al., 1983). A wide variety of bacteria can be cultured from the uteri of early postpartum cows. Sagartz and Hardenbrook (1971) found that histopathological and bacteriological examination of the uterus were more reliable than clinical examination alone.

The most commonly found bacteria include Cornyebacterium pyogenes, coliforms, streptococci and clostridia (Olsen et al., 1984). Fusobacterium necrophorum and Bacteriodes melaninogenicus are two gram negative obligate anaerobes which also have been cultured from uterine swabs. Inflammation of the uterine endometrium has was shown to alter the length of the estrous cycle and has been associated with the presence of bacteria (Gallagher and Ball, 1980; Erb et al., 1981).

Cows with abnormal discharge following a normal parturition were 9 d later displaying first postpartum estrus than were cows without abnormal discharge. These cows were also delayed to first service and had more days open than did their normal counterparts (Oltenacu et al., 1983). As with inflammation, only C. pyogenes has been correlated with the appearance of abnormal discharge (Hartigan et al., 1974).

Immediately following parturition, no bacteria are found in the uterus until approximately 2 d postpartum. This supports the hypothesis that the uterus is sterile at the time of calving (Eduvie et al., 1984).

It has been estimated that 85 to 90% of all cows acquire some form of uterine infection before 2 wk postpartum. However, only 38% of cows have abnormal uterine discharge by 6 wk postpartum, and between weeks 7 to 9 postpartum, less than 9% of cultured cows showed signs of abnormal discharge. Miller et al. (1980) reported that 26% of these abnormal discharges were infectious.

Septic conditions of the uterus following parturition often become worsened because of manual assistance during calving and the retention of placental membranes. It appears that these early postpartum uterine infections, if cleared before the first insemination, do not effect subsequent fertility (Griffen et al., 1974b). However, 10% of all metritic cows have been found to become anestrual (Martinez and Thiber, 1983).

Constant fluctuations in uterine flora populations were found during the first 7 wk postpartum. This would eliminate the usefulness of one time uterine samplings in helping to predict subsequent fertility of the cow (Griffen et al., 1974a). However, there were patterns in the presence of uterine inflammation and discharge which relate to day postpartum or day of the estrous cycle. Gallagher and Ball (1980) observed a slight increase in uterine infections in association with cervical opening at the time of estrus. Additionally, the presence of neutrophils, which serve as defense against infection, varied with day of the estrous cycle.

The presence of a uterine infection combined with postpartum and lactational stress is conducive to the development of pyometra. During the course of this condition serum progesterone concentrations increase, uterine fluid volume increases, uterine horn diameter increases and the cow becomes anestrual (Mortimer et al., 1983). The increase in progesterone causes a decrease in leukocytic infiltration of the uterus. Therefore, the cow's natural defense mechanism is decreased (Olsen et al., 1984). Fonseca et al. (1983) reported that 50% of all prolonged luteal phases occur in association with uterine infection. While pyometra is not a systemic illness it is probably brought about by a combination of pathogenic and endocrine factors.

Isolation of C. pyogenes in cows which subsequently developed pyometra increased with time until approximately 17 d postpartum and then occurrence remained constant. This bacteria was associated with severe endometritis. Increase in C. pyogenes was accompanied by increasing amounts of B. melaninogenicus and F. necrophorum. In cows with retained fetal membranes which did not develop pyometric conditions, the gram negative anaerobes initially increased until approximately d 13 postpartum at which time the presence of these bacteria decreased. The incidence of these bacteria in cows without retained placenta that did not develop pyometric conditions also decreased with time postpartum (Ruder et al., 1981).

The presence of C. pyogenes following d 21 postpartum often leads to severe endometritis and decreased fertility to the first service postpartum. Fertility to subsequent services is not affected (Griffen et al., 1974b). Several other aerobic bacteria occur in the uterus during the postpartum period, but no correlation was found between their occurrence and subsequent fertility.

Animals which develop pyometra had higher progesterone concentrations and an earlier ovulation following parturition than their non-pyometric counterparts. A possible model for the development of pyometra involves a synergistic relationship between C. pyogenes and the anaerobes F. necrophum and B. melaninogenicus. The combination of these three microflora reach a heavy growth stage at approximately the same time as the first postpartum ovulation in cows which later develop pyometra. Under inflammatory influence of bacteria the uterus produces a purulent exudate which inhibits the formation or transportation of prostaglandins from the uterine endometrium. During a normal estrous cycle prostaglandins are transported to the ovary to cause regression of the CL. This is followed by rapid follicle maturation and ovulation. Abnormal prostaglandin transport leads to an extended luteal phase and ensuing pyometra. Incidence of the gram negative anaerobes eventually declines. However, their influence has allowed for the establishment of C. pyogenes which causes the pyometra to continue. Progesterone concentrations during the prolonged luteal phase of pyometra are indicative of a functional CL. (Olsen et al., 1984).

Histology of the Uterus

Repeated uterine biopsies do not adversely affect reproduction in the cow (Manspeaker et al., 1984). However, the perception of usefulness of this technique in evaluation of reproductive health differs among researchers. Sagartz and Hardenbrook (1971) did not report a high correlation between histological and bacteriological findings in infertile cows. Studer and Morrow (1978) reported a correlation between purulent exudate and bacterial isolation, along with a correlation of uterine discharge and biopsy evaluation of uterine inflammation and scarring.

Lymphocytes and neutrophils are blood constituents which play distinct roles in inflammatory reactions. Both types of cells are found in circulating blood, however, their numbers increase in the presence of pathological conditions.

Neutrophils are the first cells to aggregate in an area of infection. They serve the dual role of primary diffense and initiating further inflammatory response mechanisms. Once neutrophils have reached an affected area they are capable of phagocytizing and killing bacteria. (Robbins and Cotran, 1979).

Lymphocytes appear later than neutrophils during the course of an infection, usually during the chronic phase. Unlike neutrophils, they are not phagocytic, but do produce large amounts of antibodies (Swenson, 1984). Initally, lymphocytes are diffuse in nature, spreading evenly through infected tissue. With time, focal pockets of this blood constituent form in uterine tissue (Hartigan et al., 1974).

During estrus, inflammation of uterine endometrium and increased population of neutrophils is a normal occurrence. Therefore, biopsies taken during this phase of the reproductive cycle may appear to be acutely inflamed, while this is only a transitory response (Manspeaker et al., 1984). However, elevated neutrophils during the luteal phase of the cycle are indicative of a pathological condition (Studer and Morrow, 1978).

Modification of periglandular connective tissue results in endometrial scarring also known as periglandular fibrosis. Manspeaker et al. (1984) found varying degrees of fibrosis in 97% of first calf heifers biopsied between 30 and 35 d postpartum. Similar results were reported in samples taken from cows of varying ages with 81% of all cows in a herd showing some degree of scarring. There was a correlation between scarring and services per conception, especially as the degree of fibroblasts increased. Studer and Morrow (1978) reported slightly different findings with no correlation between biopsy evaluation and services per conception, but a significant correlation with days open. Manspeaker et al. (1984) concluded that cows with a high degree of fibrosis had poor success at maintaining pregnancy.

Histological studies of uterine biopsies have revealed that C.pyogenes was the only bacteria which showed a correlation with endometritis. Sagartz and Hardenbrook (1971) found an association between severe endometritis and the isolation of C. pyogenes. A decrease in C. pyogenes and concurrent healing of the endometrium of the uterus is often associated with an increase in the presence of other microflora. These, however, have not been shown to interfere with the healing process or subsequent fertility (Griffen et al., 1974b).

Griffen et al. (1974a) found that initial reaction to C. pyogenes involved heavy infiltration of the uterine endometrium by neutrophils. This was followed by invasion of lymphocytes and other blood constituents. Following regression of neutrophil and diffuse lymphocyte populations, focal groups of lymphocytes often remained for several weeks. Fibrosis was indicative of severe endometrial damage in this study.

The presence of lymphocytes is normally low during the entire estrous cycle. Therefore, any increase in their occurrence is considered pathological (Studer and Morrow, 1980).

Medicinal Regimens

Many different medicinal regimens have been suggested for treating uterine infection. Results from these treatments are variable and they are generally thought to be ineffective (Bretzlaff et al., 1982). Bouters and Vandelplassche (1977) indicated that treatment of the uterus with disinfectants may actually decrease a cows natural defenses by decreasing phagocytic activity of uterine polymorphonuclear neutrophils.

Infusion of the uterus with an irritating substance 3 to 4 d before estrus can lead to a shortened cycle while treatment immediately following estrus can cause the cycle to be lengthened. Additionally, the presence of blood, pus and tissue debris in the uterus significantly reduces the effectiveness of some microbial agents.

Only 60%, 44% and 37% of microorganisms cultured showed sensitivity to the commonly used tetracycline, penicillin and triple sulfa drugs, respectively. The most effective antibiotic for treatment of commonly occurring uterine bacteria was chloramphenicol (Bretzlaff et al., 1982). Unfortunately, this drug is no longer approved for use in food animals.

Bostedt et al. (1980) reported that treatment of cows with GnRH 10 to 12 d following calving leads to a reduction in uterine infection. However, neither Kesler et al. (1978) nor Nash et al.(1980) found a decrease in uterine infections in cows injected with 100 to 250 ug of GnRH between d 13 and d 15 postpartum.

Nutritional Effects on Reproduction

Dietary crude protein (CP) was shown to effect reproduction (Jordan and Swanson, 1979). While cows fed 19.3% CP had more estrous cycles before the first imsemination than cows fed 16.3 or 12.7% CP (2.5 vs 1.7), the number of services per conception decreased with decreasing amounts of CP in the diet.

Butler and coworkers (1981) reported that the first postpartum ovulation in dairy cows normally occurs approximately 10 d following the beginning of an increase in energy balance. Restricting dietary intake in beef cows decreased plasma progesterone concentrations and either increased or did not change LH concentrations (Hill et al. 1970). Cows producing greater than 30 kg/d of milk fed a diet of adequate energy but low in protein (<12.7 % CP) had lower mean serum LH concentrations than those fed either 16.3% or 19.3% CP(1.1 vs 1.3 ng/ml). However, the animals receiving 12.7% CP had higher serum progesterone during the first observed postpartum estrus cycle and fewer days open than those fed 16.3% or 19.3% CP.

Cows which were fed 19.3% CP had peaks of LH in response to exogenous GnRH that were nearly twice as large as those cows fed 16.3 or 12.7%. Similar findings were reported by Folman et al. (1973) in dairy cows. These findings suggest that high concentrations of CP in the diet may inhibit postpartum fertility by depressing progesterone concentrations despite elevated serum LH concentrations.

Blauwiekel et al. (1986) found a tendency towards decreased amplitude of LH response to GnRH in cows fed 24% CP vs 16% CP. However, there was no effect of diet on mean serum LH, pituitary LH, pituitary GnRH receptors or pulsatile LH release patterns. These findings indicated that dietary CP does not illicit its effects on reproduction via hypothalamic-pituitary control of LH release.

Edwards et al.(1980) reported a non significant increase in services per conception and calving interval as CP was increased in the ration of dairy cows. In contrast, Folman (1984) reported 1.79 services per conception in cows fed 16% CP compared to 2.25 services per conception in cows fed 20% CP. Additionally, conception rate decreased from 69% to less than 44% as CP increased from 16 to 20%.

Rations greater than 16% CP are possibly detrimental to fertility by creating a uterine environment which is not conducive to fertilization and leads to increased early embryonic mortality (Jordan et al., 1983). A high producing dairy cow requires dietary concentrations of CP well in excess of 16% to meet her nutritional requirements during early lactation. Excess ammonia created by a diet high in CP (>20%) is not completely converted to urea by the liver or may diffuse directly into the peripheral circulation from the intestines, bypassing the liver altogether. Uterine concentrations of urea are elevated by high CP diets and uterine ammonia concentrations may be as well. Excessive concentrations of these metabolites have been shown to inhibit the citric acid cycle of sperm cells and impair LH receptors on the CL. However, other researchers found no difference in reproductive performance of cows fed from 12 to 20% CP (Howard et al., 1985).

Although high dietary CP may decrease fertility, a low nutritional plane is also detrimental to reproduction. Loss of weight after calving may increase postpartum anestrous and days open by slowing the onset of ovarian activity following calving (Wettemann et al., 1985). Selk et al. (1985) reported that plasma glucose concentrations at the time of breeding, not weight loss, were related to conception rate. Beef cows with normal plasma glucose concentrations had a significantly higher conception rate during the first 12 wk postpartum, regardless of weight loss, than cows with low plasma glucose concentrations. Downie et al. (1976) reported that cows with declining body weight and increasing blood glucose were fertile while cows with both declining weight and declining glucose tended to be infertile.

Glucose concentrations have been shown to vary with the estrous cycle in species other than the cow. Dunn et al. (1972) found that glucose increased from 60.9 mg/100 ml at estrus to 68.0mg/ml at late diestrus in the sheep. This difference can probably be explained by the ewe consuming more feed during estrus than diestrus. Although a negative correlation occurred between progesterone and glucose in this study, a cause and effect relationship could not be drawn from the data. However, Jordan et al. (1983) found plasma glucose to vary with the estrous cycle, being lower at d 5 of the cycle than d 15.

CHAPTER III

MATERIALS AND METHODS

Selection of Cows

Sixty Holstein cows were divided into three groups consisting of cows which had retained placental membranes for more than 24 h following calving (RP) (n=20), cows with a purulent uterine exudate on d 15 postpartum indicative of uterine infection (UI) (n=22) and cows which did not exhibit clinical reproductive abnormalities by d 15 postpartum (C) (n=18).

Animals classified as RP received two intrauterine oxytetracycline boluses on d 2, 4 and 6 postpartum. The boluses consisted of gelatin capsules filled with a Polyotic Water Mix (American Cyanamid) Each capsule was filled with 30 g of a mix containing .5 mg of oxytetracycline per g. Therefore, each treatment of 2 boluses contained approximately 3 g of oxytetracycline. No further treatment for reproductive abnormalities was administered to any of the cows for the course of the experiment. No animals received antibiotics other than intramammary mastitis treatment.

On d 15 postpartum animals which previously had been classified RP were injected intramuscularly with either 200 ug GnRH (CEVA Laboratories, Overland Park, KS), or 4 cc sterile saline (Baxter Laboratories Inc.; Morton Grove, IL). A jugular blood sample was drawn into a heperanized syringe and a KMAR® heat mount detector (KMAR Inc., Steamboat Springs, CO) was applied.

The remaining cows were evaluated for assignment to either UI or C groups on d 15 postpartum. The perineal area was washed with an iodine scrub preperation and the tail secured over the cow's back. A vaginal speculum consisting of 5 cm PVC pipe approximately 45 cm long was inserted into the vagina as far as the cervix. Using a flashlight, the posterior genital tract and exterior cervical os were examined for the presence of abnormal discharge, clear mucus or lochia (normal red-brown discharge caused by breakdown of the caruncular stalk). Only animals with definitely abnormal discharge or clear mucus on d 15 postpartum were used. The vaginal speculum was used in the same manner three times per week to evaluate uterine discharge between wk 2 and 7 postpartum. Following assignment to the UI or C group, these cows were treated in the same manner as the RP group.

Clear mucus was assigned a vaginal discharge score of one. Mucus that was cloudy or flecked with pus received a score of two, while a score of three was given to cows with a thick white discharge. An "off color", thickened , red-brown discharge was given a score of four.

Collection of Data

Beginning on d 16 postpartum the cows were palpated 3 times weekly per rectum to determine cervix diameter (mm), uterine horn diameter (mm), uterine fluid volume (ml), and the type and nature of ovarian structures. Follicular size was recorded as well as the presence of CL. If a CL was firm, suggesting regression, this was noted. Differentiations were made between CL and corpus hemmorhagicum. Follicles greater than 25 mm were considered cystic.

Uterine swabs and uterine tissue biopsies were collected once per week between d 15 and 50 postpartum. These samples were evaluated by Virginia Maryland Regional Veterinary College Diagnostic Clinic. The perineal area of the cow to be sampled was washed with an iodine solution. A sterile modified Tiegland swab was introduced into the vagina after the vulvar lips had been spread to prevent possible contamination of the swab. Placement of the swab into the uterus was performed by cervical manipulation through the rectum. After the uterine swab sample had been obtained the swab was retracted into its protective sheath, removed from the reproductive tract and further retracted to allow removal of the contaminated sheath end. The swab and its sheath were then placed in a sterile plastic bag and the ends capped until evaluation for aerobic bacteria.

Uterine swabs were streaked on a 5% sheep blood agar plate. Blood agar serves as a growth medium for fastidious heterotrophs and allowed determination of various hemolytic patterns. If gram negative rods were cultured from the blood agar plate, they were transferred to MacConkey's medium for further evaluation.

Only gram negative enteric rods will grow on MacConkey's medium. Gram positive bacteria are inhibited by the inclusion of bile salts. Additional identifications can be made from a MacConkey's agar plate by the ability of a microorganism to use lactose fementation as an energy source, in which case, the colony will take on pink coloration. Non-lactose fermenters are amber when grown on MacConkey's agar. MacConkey's is useful for positive identification of <u>Escherechia coli</u> and <u>Klebsiella</u> species (Pelczar and Chan, 1981).

A thioglycollate tube was inoculated with the swab at the same time the blood plate was streaked. Sodium thyioglycollate medium maintains a strongly anaerobic environment deep within the medium, while developing an aerobic area near the top. Therefore, microaerophiles, such as streptococcus species, grow at the interface between the aerobic and anaerobic regions. The plates and thioglycollate tube were incubated for 24 h at 37 C and then observed for growth. If colonies were not observed, the plates and tubes were incubated for an additional 48 h. If colonies were still not evident at this time, "no growth" was said to have occurred.

Uterine biopsies were obtained in a manner similar to uterine swab collection, using a Jones Colonie biopsy instrument (Stortz Instrument Company; St. Louis, MO) which was stored in an alcohol bath. Jaws of the biopsy instrument were opened once it had been placed in the body of the uterus via cervical manipulation and pressure was applied through the rectum to allow a piece of endometrium approximately .5 by .3 cm to be excised. Bouin's fixative was used to preserve the samples for 24 to 36 h following which they were stored in 70% ethyl alcohol until histological evaluation. The frequency of neutrophils, diffuse and focal lymphocytes and fibroblasts was determined.

Blood samples were drawn by jugular venipuncture into heperanized syringes 3 times per week. These were stored on ice until plasma was collected by centrifugation. Plasma samples were stored at -20 C until assayed.

Cows were observed for estrual activity twice daily. All of the cows were bred at the first display of estrus occurring after d 50 postpartum.

Following d 50 postpartum cows were palpated once per 2 weeks to monitor ovarian activity and determine pregnancy. At d 175 postpartum or when pregnancy was confirmed, cows were removed from the study.

Determination of Plasma Urea, Glucose and Progesterone

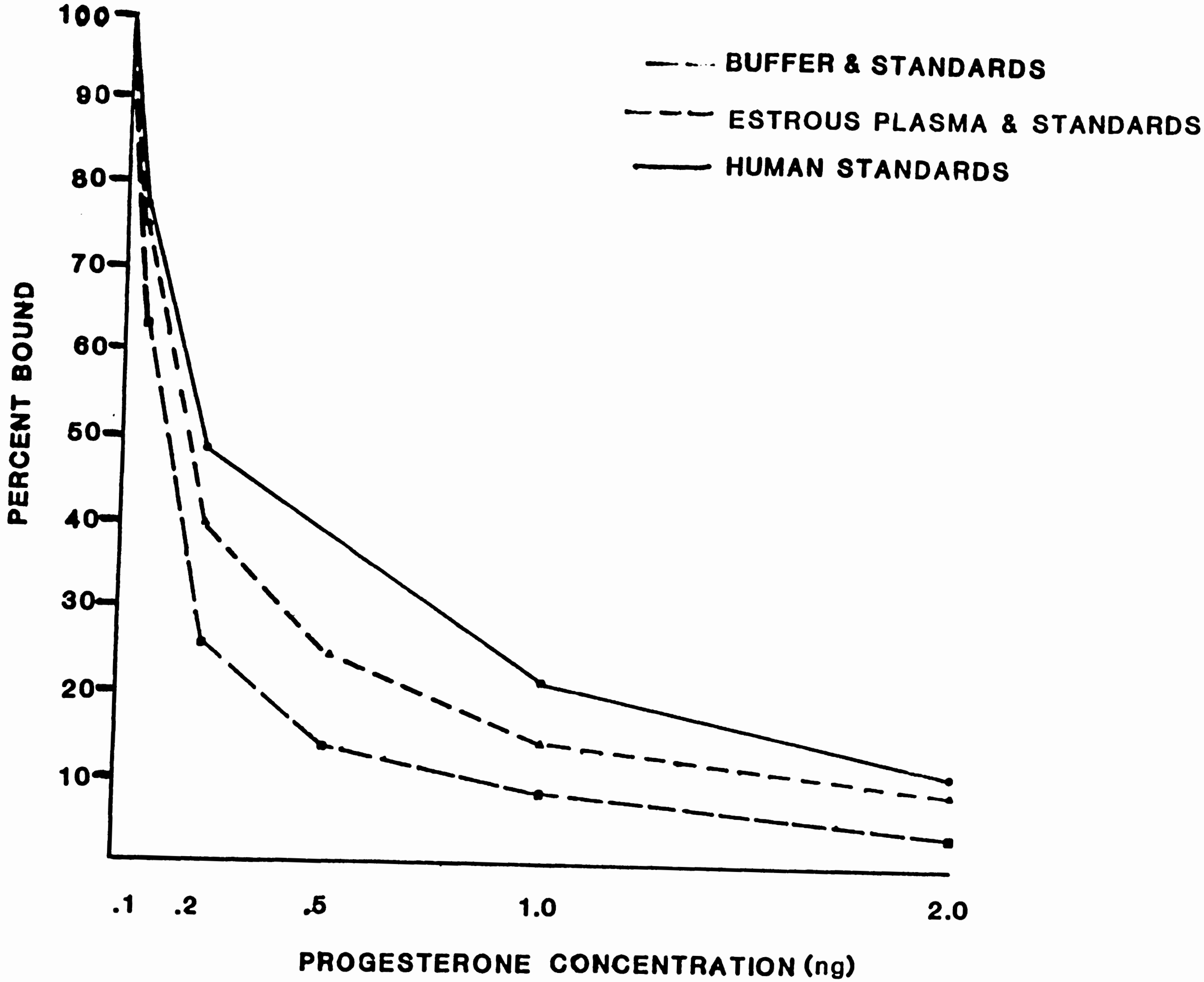
Plasma progesterone content was determined using the solid phase radioimmunoassay method (Diagnostic Products Corp.; Los Angeles, CA). This procedure was modified for use with bovine plasma by comparing standard curves obtained from progesterone standards prepared in bovine plasma collected during estrus, assay buffer, or the human standards supplied with the kit. These curves were parallel between progesterone concentrations of .2 ng/ml and 2 ng/ml (Fig 1). However, the amount of radioactive hormone bound was higher in human standards than in bovine standards at similar hormone concentrations. This indicates a greater degree of sensitivity in bovine estrual plasma binding than in binding of the human standards.

Validity of this method for assaying progesterone in bovine plasma was demonstrated by adding known amounts of nonradioactive hormone to aliquots of plasma and assaying (Table 1). The values obtained were compared between the standard curves prepared in buffer, estrual plasma or human standards (Table 2). Values for standards prepared in estrual plasma showed the least variation from known progesterone concentrations. Therefore, standards prepared in bovine estrual plasma were used in the assays.

Intra-assay coeffecients of variation for the three progesterone assays were 13.9%, 8.4% and 10.7%.

FIGURE 1

PROGESTERONE STANDARD CURVES IN BUFFER, ESTROUS PLASMA OR HUMAN SERUM



BUFFER & STANDARDS
ESTROUS PLASMA & STANDARDS
HUMAN STANDARDS
PERCENT BOUND
100
90
80
70
60
50
40
30
20
10
.1
.2
.5
1.0
2.0
PROGESTERONE CONCENTRATION (ng)

Table 1. PRECISION OF PROGESTERONE DETERMINATIONS IN BOVINE PLASMA USING 'COAT-A-COUNT'[a] RADIOIMMUNOASSAY.

	Standards prepared in:		
	Buffer	Human Serum	Bovine Plasma
luteal plasma	4.33±.08[bc]	17.5±.34	6.2±.14
estrual plasma	.20±.0	.92±.04	.13±.02

[a]Diagnostic Products Corp. Los Angeles, CA

[b]Expressed in ng/ml±standard error.

[c]n=6

Table 2. RECOVERY OF ADDED UNLABELED PROGESTERONE FROM BOVINE LUTEAL AND ESTROUS PLASMA ASSAYED AGAINST BUFFER, HUMAN SERUM AND BOVINE ESTROUS PLASMA PREPARED STANDARDS.

Progesterone Added (ng)	Progesterone Measured (ng)					
Estrous plasma	Buffer	SE	Human Serum	SE	Bovine Plasma	SE
0.0[a]	.02	.01	.07	.004	.02	.00
0.05	.06	.00	.22	.002	.05	.00
0.2	.19	.00	.71	.005	.24	.00
1.0	.80	.30	3.44	.263	1.23	.10
2.0	1.47	.18	6.74	.903	2.64	.43
5.0	1.00	.05	4.42	.197	1.62	.08
Luteal plasma						
0.0	.28	.02	1.11	.31	.39	.02
0.05	.30	.00	1.19	.02	.42	.00
0.2	.41	.03	1.64	.67	.58	.05
1.0	1.14	.00	5.07	.02	1.88	.01
2.0	1.71	.25	7.95	.89	3.25	.47
5.0	1.37	.08	6.21	.04	2.38	.17

[a]n=duplicates

Glucose content of the plasma (mg/dl) was determined using the O-toludiene method of Feteris (1965). Plasma urea (mg/dl) was quantified by the diactyel monoxamine and thiosemicarbizide procedure of Coulombe and Farreau (1963).

Statistical Analysis

The effect of clinical group, treatment, days postpartum, days postpartum squared and days postpartum cubed as well as interactions of these on ovarian structures, uterine and cervical diameter, vaginal discharge, uterine fluid volume and plasma glucose, urea and progesterone content were evaluated. This model was tested by analysis of variance using cow (treatment x clinical group) as the error term for treatment, clinical group and the interaction of these two.

Services per conception, days open and conception rate were evaluated using a model which contained clinical group, treatment group, the interaction of these two, milk yield and age as independent variables.

Chi-square analysis was used to determine changes in uterine histology and bacteria content over time postpartum as well as between the various clinical and treatment groups. Analysis of variance was also used to determine possible clinical, treatment, or time postpartum effects on these variables.

All variables examined in this study were compared in a correlation table. Only those correlations which were reasonable were presented.

CHAPTER IV

RESULTS AND DISCUSSION

Vaginal score, uterine volume and reproductive tract diameter

Assignment of cows to a clinical group based on RP at 24 h postpartum or nature of uterine discharge on d 15 postpartum was accurate. The vaginal discharge score was different between the three clinical groups ($P<.01$) with C cows having a discharge which was significantly more clear than that of UI or RP cows (Table 3). Oltenacu et al. (1983) reported a correlation between abnormal parturition and vaginal discharge. Although calving difficulty was not evaluated in this study, the incidence of retained placenta was associated with an increase in vaginal discharge score. Cows with RP or UI had mean vaginal discharge scores of 2.51±.04 and 2.60±.04 (X q SE), respectively. This was indicative of discharge that fluctuated between cloudy and flecked with pus (2.00) to being a thick, purulent discharge (3.00). The discharge score of C cows was 1.67±.06 (X q SE) representing a fluctuation between clear (1.00) and cloudy or pus flecked (2.00). Additionally, nature of the discharge changed with time postpartum in a linear manner (Fig. 2). A high score of 2.7 in RP cows indicated a thick discharge was characteristic at d 15 postpartum. The score decreased slightly, to 2.5 at 50 d postpartum.

Uterine infection at d 15 postpartum was associated with a score of 3.0, somewhat higher than that seen in RP cows. This decreased in a linear manner to a score of 2.2 at d 50. The decrease between d 15 and 50 postpartum of vaginal discharge in UI cows indicated improvement in

Table 3. ANALYSIS OF VARIANCE FOR VAGINAL DISCHARGE SCORE AND UTERINE VOLUME BY TREATMENT (T), CLINICAL GROUP (CG) AND DAY POSTPARTUM (DPP).

	Vaginal Score		Uterine Volume	
Source	df	MS	df	MS
T	1	.13	1	.016
CG	2	17.82**	2	.086
T X CG	2	.09	2	.039
Cow(T X CG)	54	3.00**	54	.056**
DPP	1	2.66**	1	.155**
DPP^2	--	--	1	.130**
DPP X T	1	.42	1	.013
DPP^2 X T	--	--	1	.016
DPP X CG	2	3.90**	2	.060**
DPP^2 X CG	--	--	2	.043**
Error	693	.47	749	.006

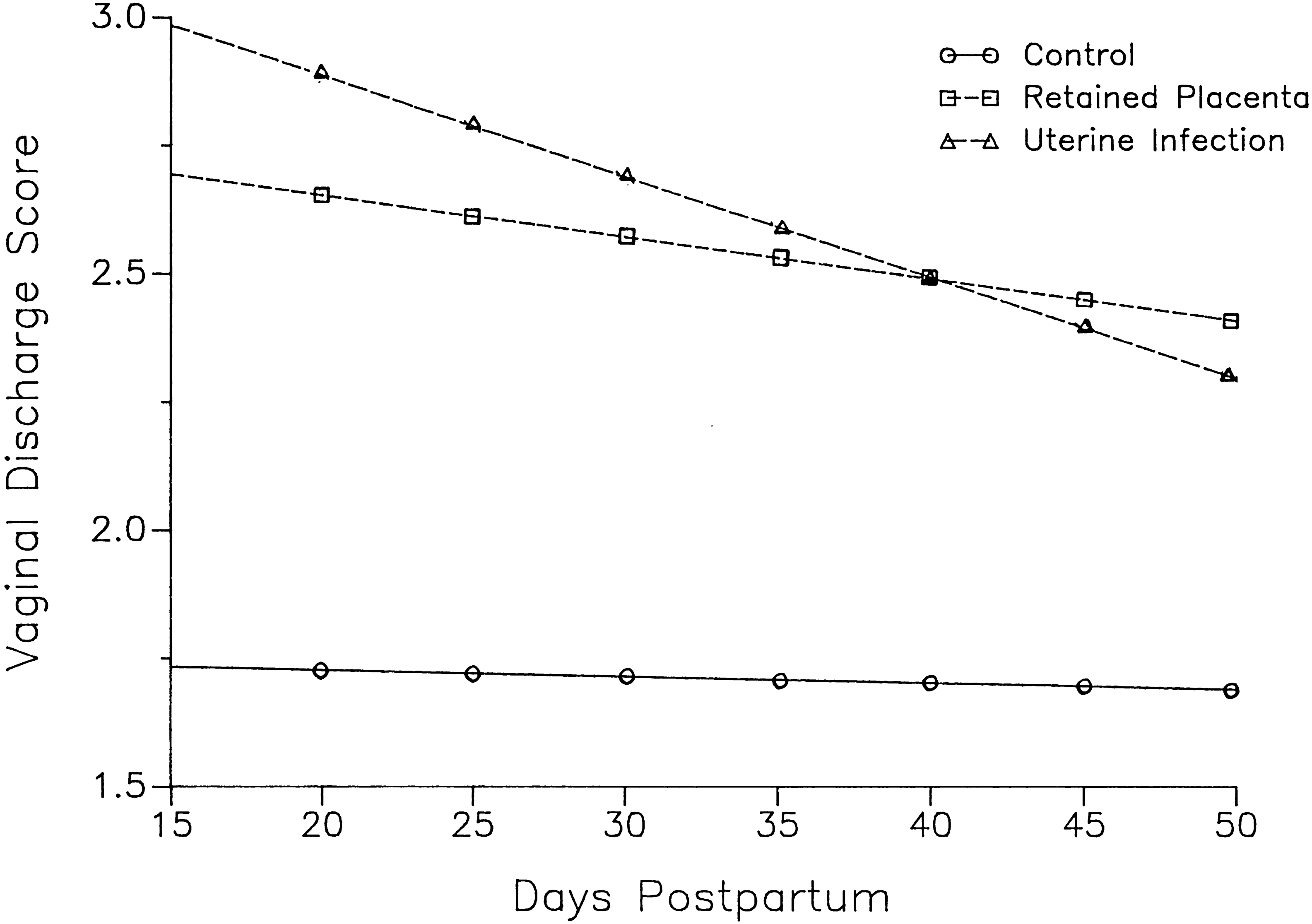
* P< .05

** P< .01

T, CG and T X CG tested by Cow(T X CG).

FIGURE 2

VAGINAL DISCHARGE SCORE IN COWS WITH RETAINED PLACENTAS, UTERINE INFECTION AT D 15 OR WERE CONTROL COWS, OVER TIME



Control
Retained Placenta
Uterine Infection
Vaginal Discharge Score
3.0
2.5
2.0
1.5
15
20
25
30
35
40
45
50
Days Postpartum

uterine health even though the cows were not treated with antibiotics. The initial score of 3.0 in this group tended to be greater than the score of 2.7 for RP cows. However, by d 50 postpartum UI cows had a vaginal discharge score lower than that of RP cows. It appears that occurrence of uterine conditions which illicit purulent discharge are more prolonged in RP than UI cows.

Vaginal discharge scores of C cows were lower than those of RP or UI cows. A mean initial score of 1.73 was found at d 15 indicating a cloudy discharge. This dropped to 1.69 by d 50 postpartum indicating that discharge did not change dramatically over time postpartum. From these findings it appears that uteri not having a discharge at d 15 postpartum remained without a discharge to d 50 postpartum.

Neither treatment with GnRH nor clinical condition affected involution of the reproductive tract (Table 4). However, size of the tract changed in a curvilinear manner over time postpartum.

In all three clinical groups, cervical, left horn and right horn diameters reached a nadir at 40 d postpartum followed by a slight increase between d 40 and 50 postpartum. The largest initial right horn diameter of 46.5 mm was seen in the RP group (Fig. 3). This decreased to 31.2 mm by d 40 and increased to 33.5 mm at d 50 postpartum. Cows in the UI group had an initial right horn diameter of 36 mm, a minimum at d 40 postpartum of 28.5 mm and a slight increase to 29.5 mm at d 50 postpartum. Right horn diameter of the C group at d 15 postpartum was intermediate between that of RP and UI cows. An initial diameter of 40.2 mm at d 15 decreased to 30.5 mm at d 40 and increased 32 mm by d 50 postpartum. Therefore, the initial right horn diameter ranged over 10 mm from a low of 36 for

Table 4 . ANALYSES OF VARIANCE FOR LEFT HORN, RIGHT HORN AND CERVIX DIAMETER BY TREATMENT (T), CLINICAL GROUP (CG) AND DAY POSTPARTUM (DPP).

Source	df	Left Horn Diameter MS	Right Horn Diameter MS	Cervix Diameter MS
T	1	12	56	26
CG	2	123	345	341
T X CG	2	100	140	71
Cow(T X CG)	54	106**	409**	349**
DPP	1	1667*	3621**	5059**
DPP^2	1	1224*	2210**	3375**
DPP X T	1	10	14	8
DPP^2 X T	1	7	12	6
DPP X CG	2	101**	429**	479**
DPP^2 X CG	2	79**	308**	341**
Error	714	13	21	22

* P<.05

** P<.01

T,CG, and T X CG tested by Cow(T X CG).

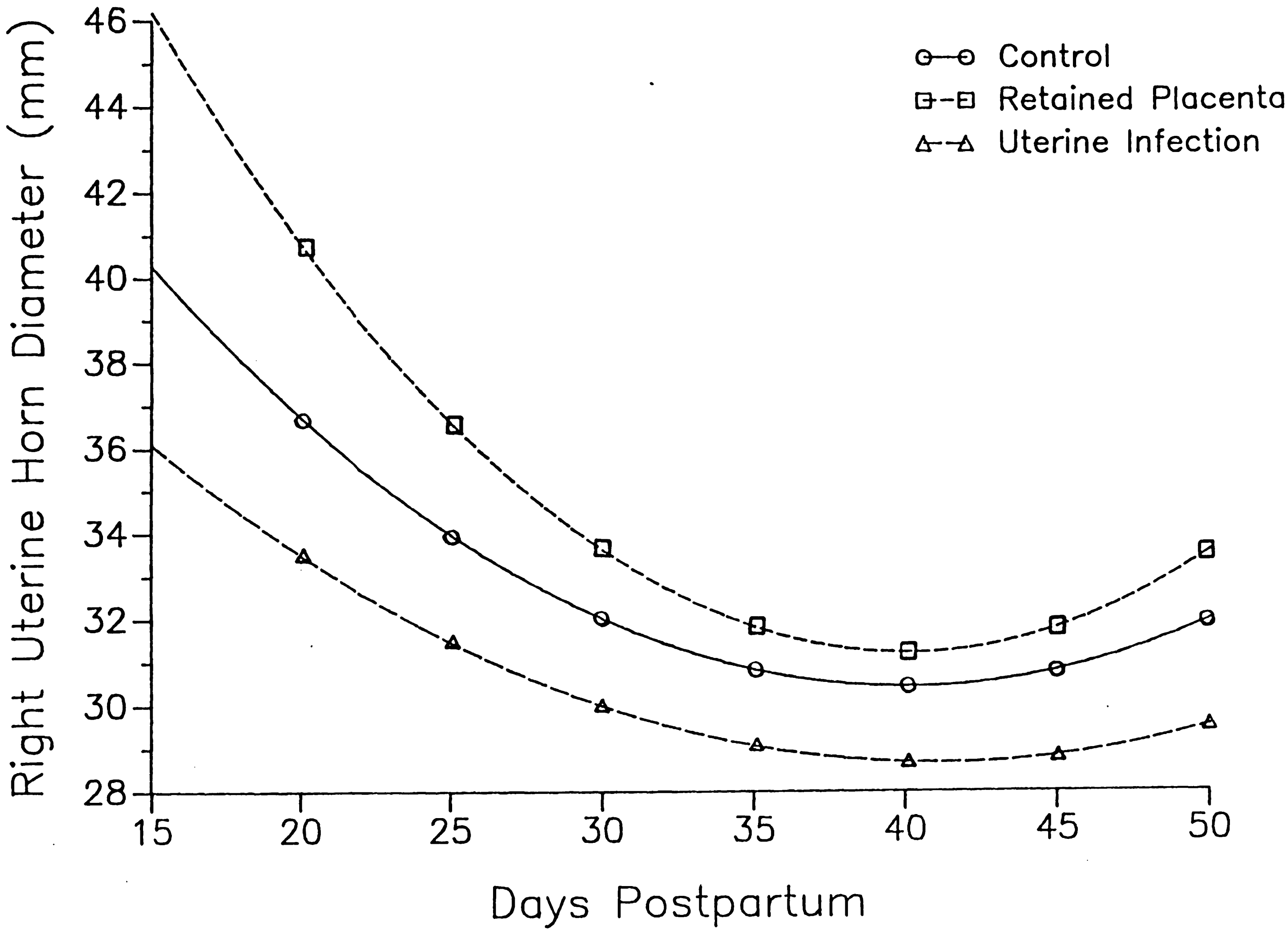


FIG. 3. RIGHT UTERINE HORN DIAMETER IN COWS WITH RETAINED PLACENTAS, UTERINE INFECTION AT D 15 OR WERE CONTROL COWS, OVER TIME

UI cows to a high of 46.5 in the RP group. However, involuted size varied little, between 28.5 and 31.2 mm.

Little difference was seen between the groups in left horn diameter (Fig. 4). Initial diameter of the left horn ranged from a low of 32.5 mm for the C group to a high of 38.5 mm for the UI group. Cows in the UI group were intermediate with an initial diameter of 36 mm. Involuted diameter of the three groups ranged from 27 mm for the UI and C groups to 27.5 mm for the RP group.

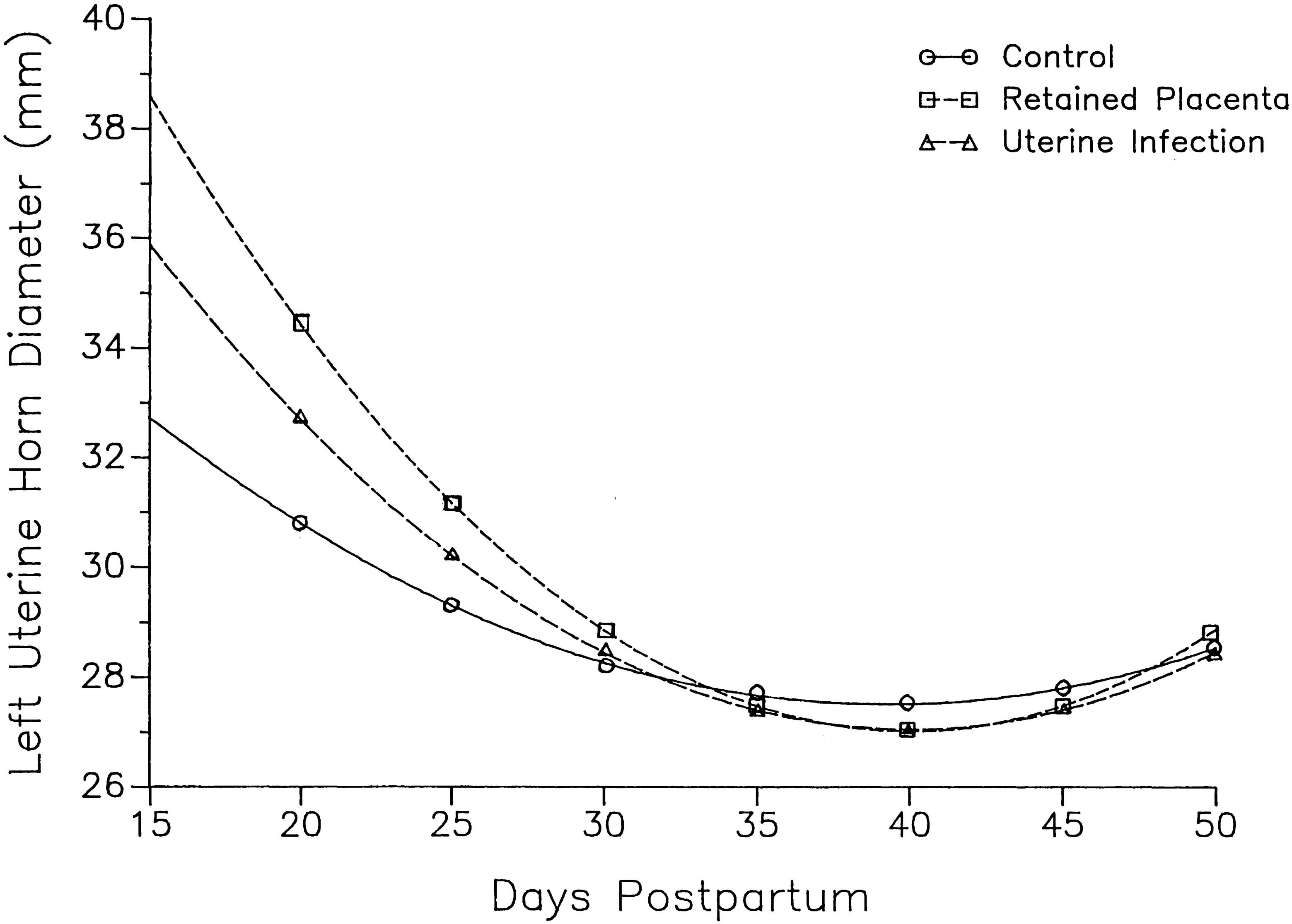
As with left horn diameter, involuted diameter of the cervix was similar for the three groups, although it showed an initial large range (Fig. 5). Cows in the RP group had an initial cervical diameter of 55 mm which decreased to 32.5 mm by d 40 postpartum and then increased to 37 mm at d 50.

Cervical diameter of animals in the UI group decreased from 42 to 29.5 mm between d 15 and 40 postpartum and then increased slightly to 31.5 mm at d 50. Control cows had a cervical diameter of 40.2 mm at d 15 which decreased to 32.3 mm at d 45 postpartum. Cervical diameter then increased very slightly to 32.5 mm at d 50 postpartum. The C cows were 5 d later to attain minimum cervical diameter than were cows in the other clinical groups. However, only a .25 mm decrease was seen in cervical diameter between d 40 and 45 postpartum. Unlike Oltenacu et al., (1983) who reported a relationship between cervix size and discharge postpartum, a more purulent discharge did not cause an increased cervix size in this study.

An effect of age on reproductive tract involution rate has been reported (Oltenacu et al., 1983; Fonseca et al., 1983). Both researchers

FIGURE 4

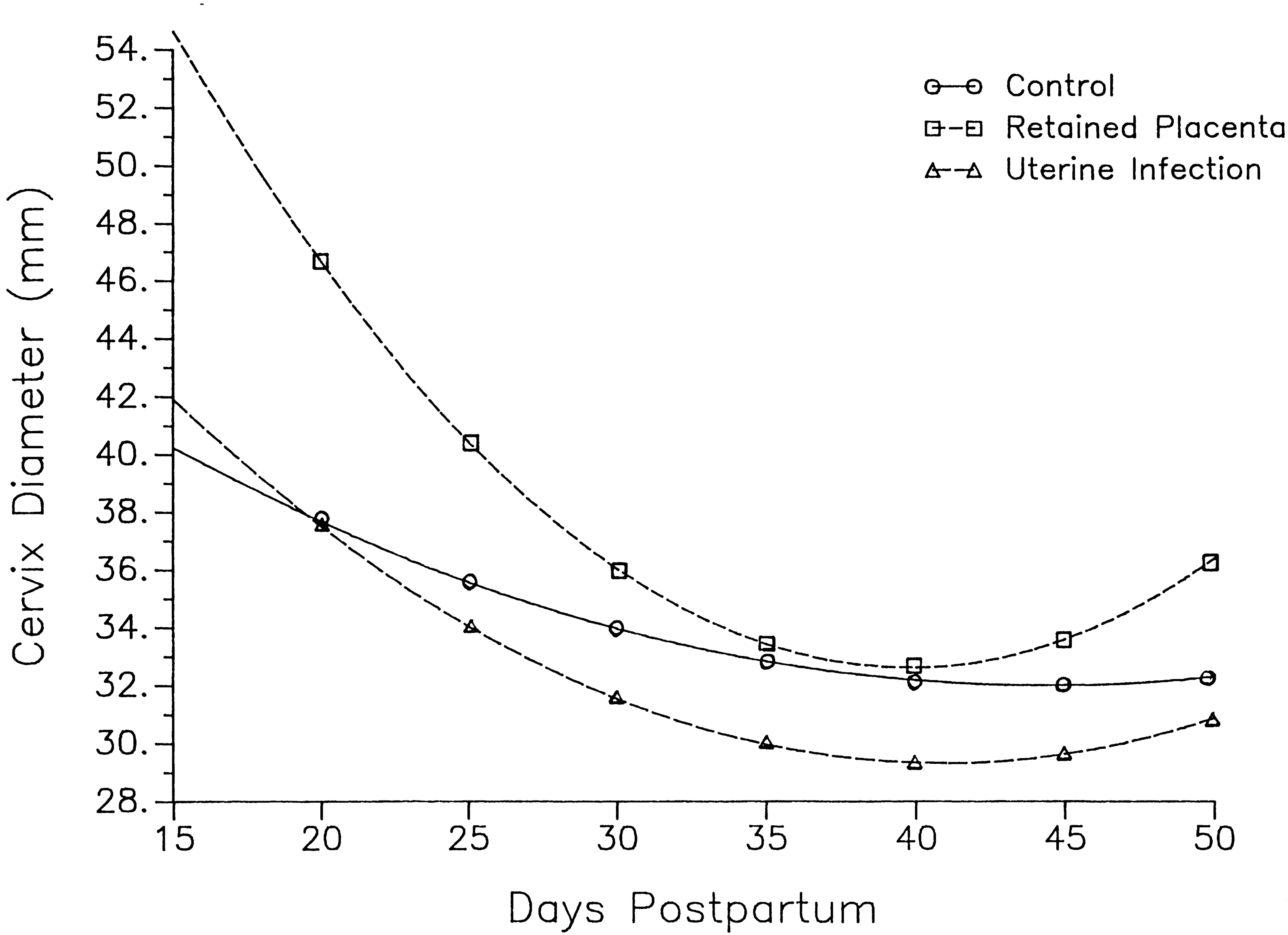
LEFT UTERINE HORN DIAMETER IN COWS WITH RETAINED PLACENTAS, UTERINE INFECTION AT D 15 OR WERE CONTROL COWS, OVER TIME



Left Uterine Horn Diameter (mm)
40
38
36
34
32
30
28
26
15
20
25
30
35
40
45
50
Days Postpartum
Control
Retained Placenta
Uterine Infection

FIGURE 5

CERVICAL DIAMETER IN COWS WITH RETAINED PLACENTAS, UTERINE INFECTIONS AT D 15 OR WERE CONTROL COWS, OVER TIME


Cervix Diameter (mm)
Days Postpartum
Control
Retained Placenta
Uterine Infection
54.
52.
50.
48.
46.
44.
42.
40.
38.
36.
34.
32.
30.
28.
15
20
25
30
35
40
45
50

reported that involution occurred sooner in young cows and cows without clinical reproductive conditions. It is possible that the affect of clinical group on involution was masked in this study by age differences between the groups (Table 5). Cows with fewer than three lactations were more likely to have RP or UI than cows with three or more lactations. Cow in the control group averaged 3.1 lactations, RP cows 2.4 and UI cows 2.0 lactations. Therefore, the tendency of a clinical condition to slow involution may have been offset by the faster involution rate seen in young cows.

The vaginal score in RP, UI and C cows continued to decrease with time postpartum despite a slight increase in reproductive tract diameter (Table 3). The increase in reproductive tract size occurred in conjunction with a slight increase in uterine fluid volume (Fig. 6). Retained placenta cows had the greatest increase in right horn size (31.2 to 33.5 mm) (Fig. 3) as well as the greatest increase in uterine fluid volume (25 to 50 ml) (Fig. 6) between d 40 and 50 postpartum. Control cows had an increased right horn diameter of 1.5 mm between d 40 and 50 postpartum, however, uterine fluid volume increase during this time was inconsequential.

Right horn diameter increased slightly from 28.5 to 29.5 mm between d 40 and 50 postpartum in the UI group (Fig. 3). Over this same time period uterine fluid volume did not change markedly, fluctuating between 28 and 28.5 ml (Fig. 6).

Unlike the findings of Griffen et al. (1974) clinical reproductive abnormalities did not increase the time to involution of the reproductive tract. Involution of the left and right uterine horns was complete by d

Table 5. LEAST SQUARES MEANS ± STANDARD ERRORS FOR LACTATION NUMBER BY CLINICAL GROUP.

Clinical Group	Lactataion Number
Retained Placenta	2.4±.3[a,b]*
Uterine Infection	2.0±.3[a]
Control	3.1±.3[b]

*Values with different superscripts are different at P<.05.

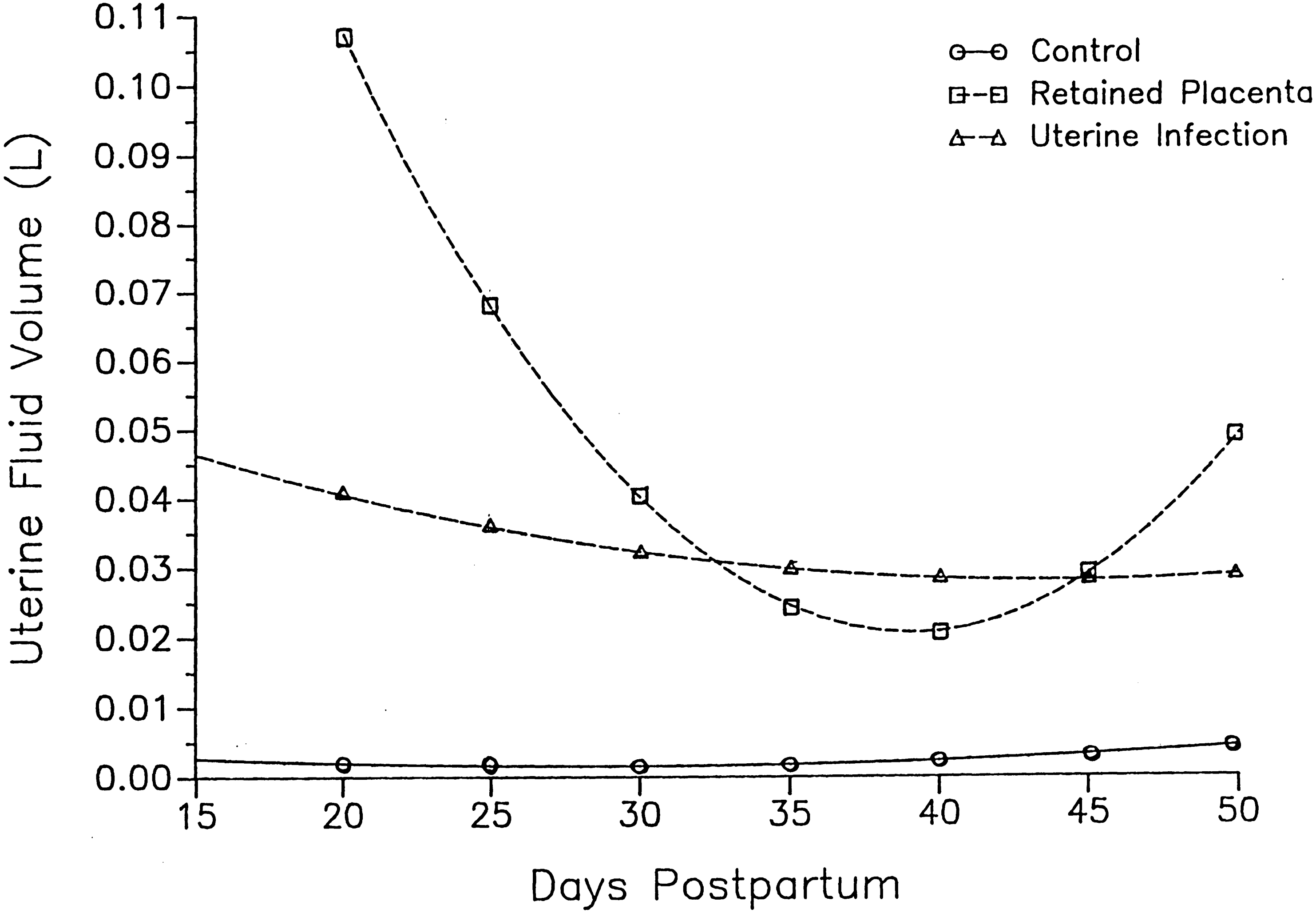


FIG. 6. UTERINE FLUID VOLUME BY CLINICAL GROUP IN COWS WITH RETAINED PLACENTAS, UTERINE INFECTION AT D 15 OR WERE CONTROL COWS, OVER TIME

40 postpartum in all groups (Fig. 3 and 4). In C cows the cervix reached a minimum diameter of 32 mm by d 45 postpartum which was 5 d later than attainment of minimum diameter in the RP or UI cows (Fig. 5). Additionally, this diameter was slightly larger than that of the UI cows, which had a minimum cervical diameter of 29 mm and was only slightly smaller than the involuted diameter of RP cows, 32.5 mm. Similarly, UI cows had the smallest involuted diameter of left and right horn (27 and 28.7 mm, respectively) at completed involution. Left and right horn diameters of the C cows were 27.5 and 30 mm, respectively (Fig. 3 and 4). Minimum values of RP cows were 27mm for the left horn and 31.5mm for the right horn. Uterine volume was lowest in the C group and therefore most likely did not cause the increased size of this group's reproductive tract in comparison with other groups. Control cows were also older than UI or RP cows (Table 5). Therefore, the reported effect of age on reproductive tract size (Oltenacu et al., 1983) most likely accounts for C cows displaying larger reproductive tracts than UI cows.

Frequency of ovarian follicles

The frequency of occurrence of ovarian follicles was not affected by clinical group. However, injection of GnRH had a significant effect of follicular frequency on the left ovary between d 15 and 50 postpartum (Table 6). A higher percentage of follicles occurred at d 15 in cows injected with saline than in GnRH treated cows (Fig. 7). Twenty-two percent of the the saline treated cows had follicles on the left ovary at d 15 postpartum while slightly less than 16% of the GnRH treated cows had follicles at this time. The percentage of cows displaying left ovarian follicles decreased over time in both treatment groups. Saline

Table 6. ANALYSES OF VARIANCE FOR FOLLICLES ON THE LEFT AND RIGHT OVARY BY TREATMENT (T), CLINICAL GROUP (CG) AND DAY POSTPARTUM (DPP).

	Follicle Right Ovary		Follicle Left Ovary	
Source	df	MS	df	MS
T	1	.04	1	1.02
CG	2	.09	2	.10
T X CG	2	.03	2	.02
Cow(T X CG)	54	.23**	54	.30**
DPP	1	.00	1	.47*
DPP^2	1	.10	--	--
DPP X T	1	.02	1	.56*
DPP^2 X T	1	.02	--	--
DPP X CG	2	.08	2	.01
DPP^2 X CG	2	.07	--	--
Error	758	.10	766	.11

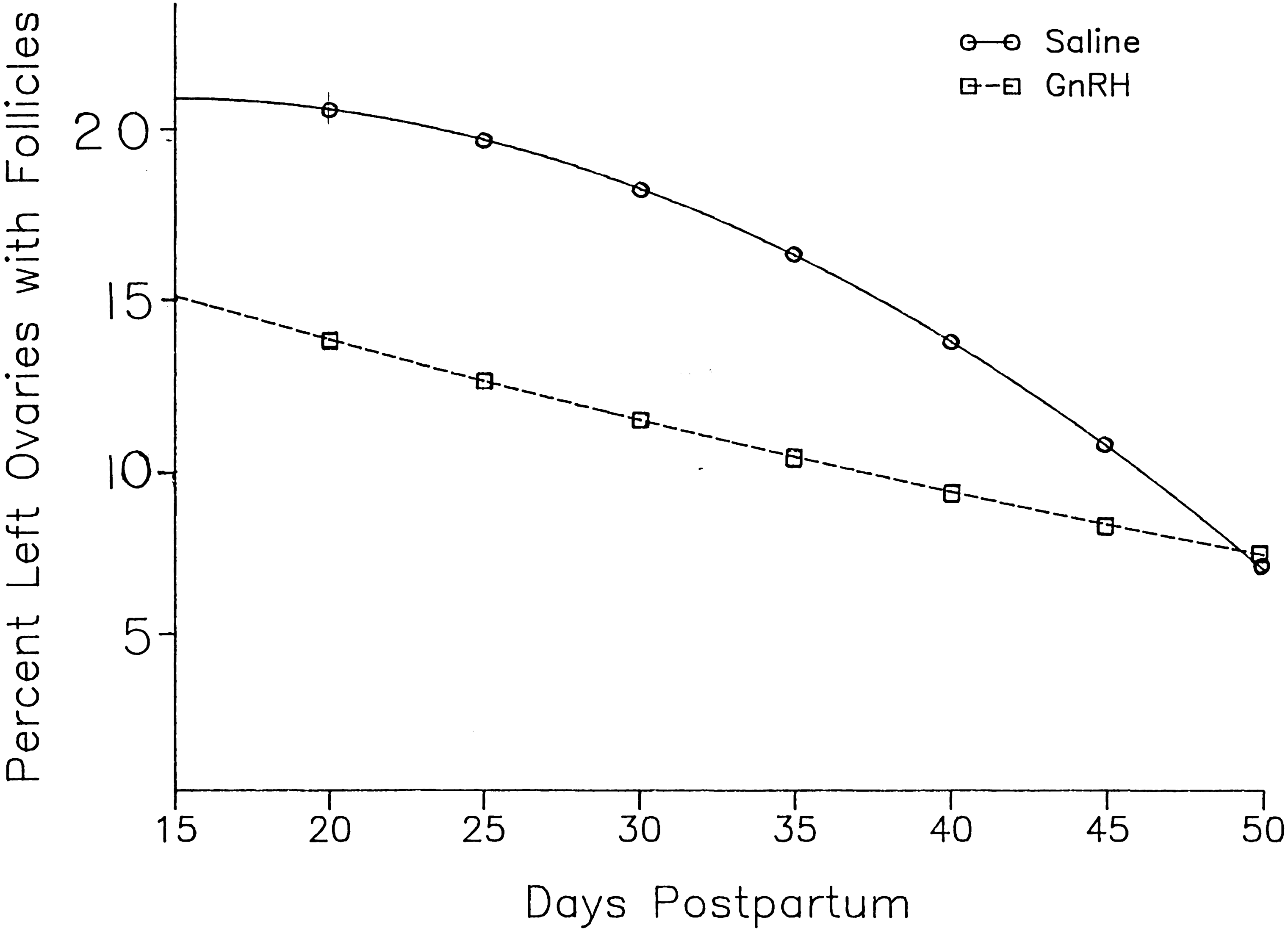
* P<.05

** P<.01

T, CG and T X CG tested by Cow(T X CG)

FIGURE 7

PERCENT LEFT OVARIES WITH CORPERA LUTEA BY TREATMENT OVER TIME


Saline
GnRH
Percent Left Ovaries with Follicles
20
15
10
5
15
20
25
30
35
40
45
50
Days Postpartum

injected cows had a drop from 22% at d 15 postpartum to slightly less than 7.5% at d 50 postpartum. Gonadotropin releasing hormone injected cows dropped from 16% to slightly less than 7% occurrence of follicles on the left ovary. It is possible that the decrease in follicular occurrence in both groups is due to an increased occurrence of CL.

Corpus luteum changes and plasma progesterone concentration.

A significant difference in progesterone patterns was seen in the three clinical groups during the first 50 d postpartum (Table 7). Additionally, frequency of CL on the right ovary was also different between the clinical groups (Table 8). In all groups progesterone concentration and right ovary CL frequency followed a curvilinear pattern (Fig. 8 and 9).

Cows which retained placental membranes had an average of 29 d after calving before progesterone concentrations were greater than 1 ng/ml, indicative of a functional CL. By 50 d postpartum progesterone concentrations had dropped to approximately 1.1 ng/ml from a peak of 1.9 ng/ml at d 40. This pattern is indicative of normal luteal function and estrous cycle length.

Frequency of CL on the right ovary was 6% in the RP group at d 15 postpartum and increased to approximately 40% by d 40 to 45 postpartum. At d 50 postpartum approximately 34% of RP cows had a CL on the right ovary.

By 27 d postpartum UI cows had reached progesterone concentrations of greater than 1 ng/ml (Fig. 8). At 50 d postpartum plasma progesterone concentrations were approximately 2.25 ng/ml having dropped only slightly from a peak of 2.5 ng/ml at 45 d postpartum. This suggests a prolonged

Table 7. ANALYSIS OF VARIANCE FOR PROGESTERONE, UREA AND GLUCOSE BY TREATMENT (T), CLINICAL GROUP (CG) AND DAY POSTPARTUM (DPP)

	Progesterone		Urea		Glucose	
Source	df	MS	df	MS	df	MS
T	1	4.0	1	101	1	442
CG	2	9.4	2	39	2	20
T X CG	2	2.4	2	743*	2	98
Cow(T X CG)	54	4.8**	54	202**	54	726**
DPP	1	.6	1	33	1	26
DPP^2	1	.1	1	60	--	--
DPP^3	1	.9	--	--	--	--
DPP X T	1	4.7	1	86*	1	251*
DPP^2 X T	1	4.7	1	84*	--	--
DPP^3 X T	1	4.5	--	--	--	--
DPP X CG	2	10.7**	2	45	2	19
DPP^2 X CG	2	10.6**	2	556*	--	--
DPP^3 X CG	2	9.8*	--	--	--	--
Error	752	2.1	744	15	738	48

* $P<.05$
** $P<.01$
T,C and T X C tested by Cow(T X C).

Table 8. ANALYSES OF VARIANCE FOR OCCURRENCE OF CORPORA LUTEA (CL) ON THE LEFT AND RIGHT OVARY BY TREATMENT (T), CLINICAL GROUP (CG) AND DAY POSTPARTUM (DPP).

	CL Right Ovary		CL Left Ovary	
Source	df	MS	df	MS
T	1	.4	1	.01
CG	2	1.0	2	.31
T X CG	2	.5	2	.11
Cow(T X CG)	54	1.0**	54	.80**
DPP	1	3.6**	1	4.42**
DPP^2	1	3.6**	--	--
DPP X T	1	.9*	1	.05
DPP^2 X T	1	1.3**	--	--
DPP X CG	2	.6*	2	.93**
DPP^2 X CG	2	.5*	--	--
ERROR	761	.2	765	.11

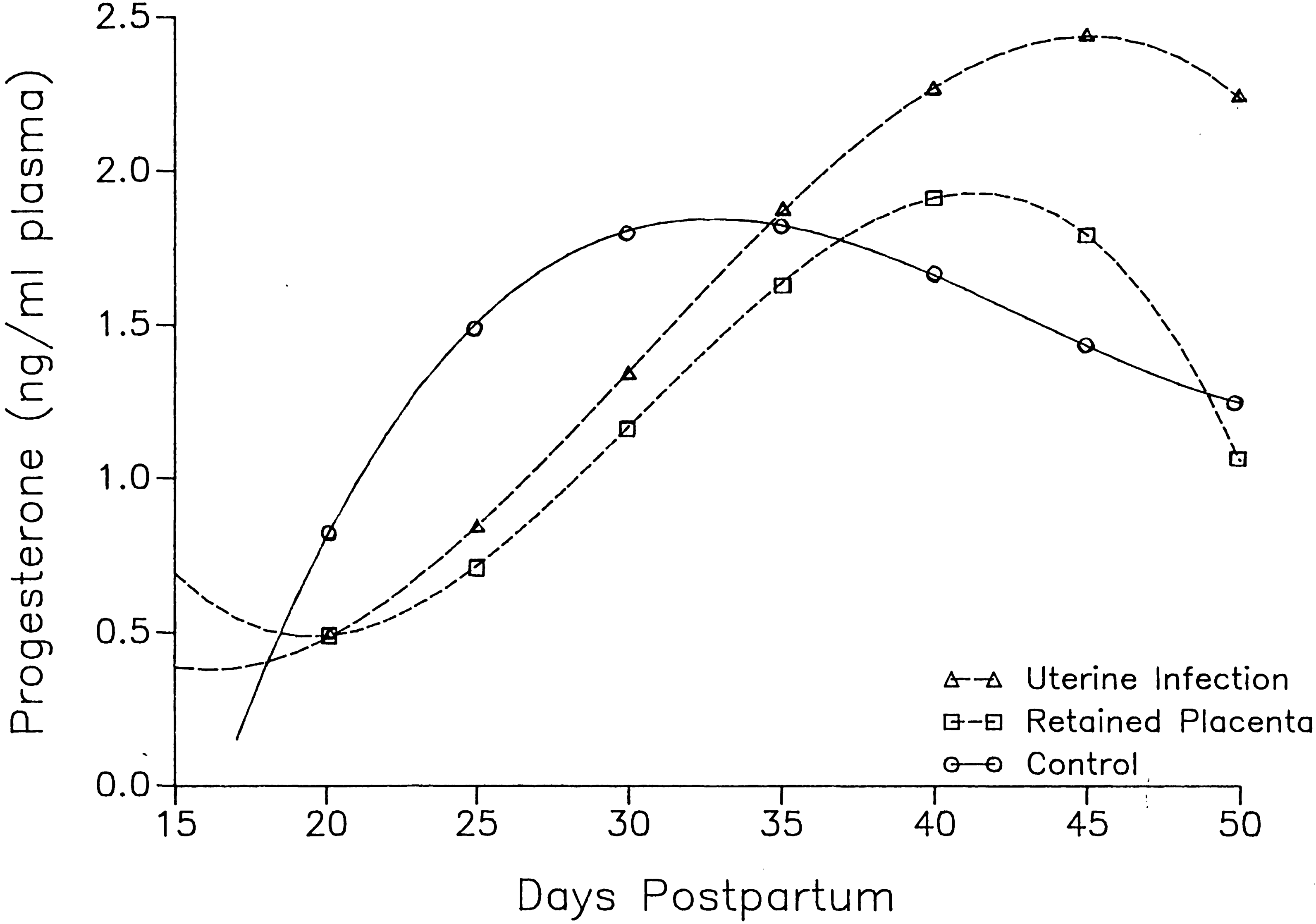
* P<.05

** P<.01

T, CG and T X CG tested by Cow(T X CG)

FIGURE 8

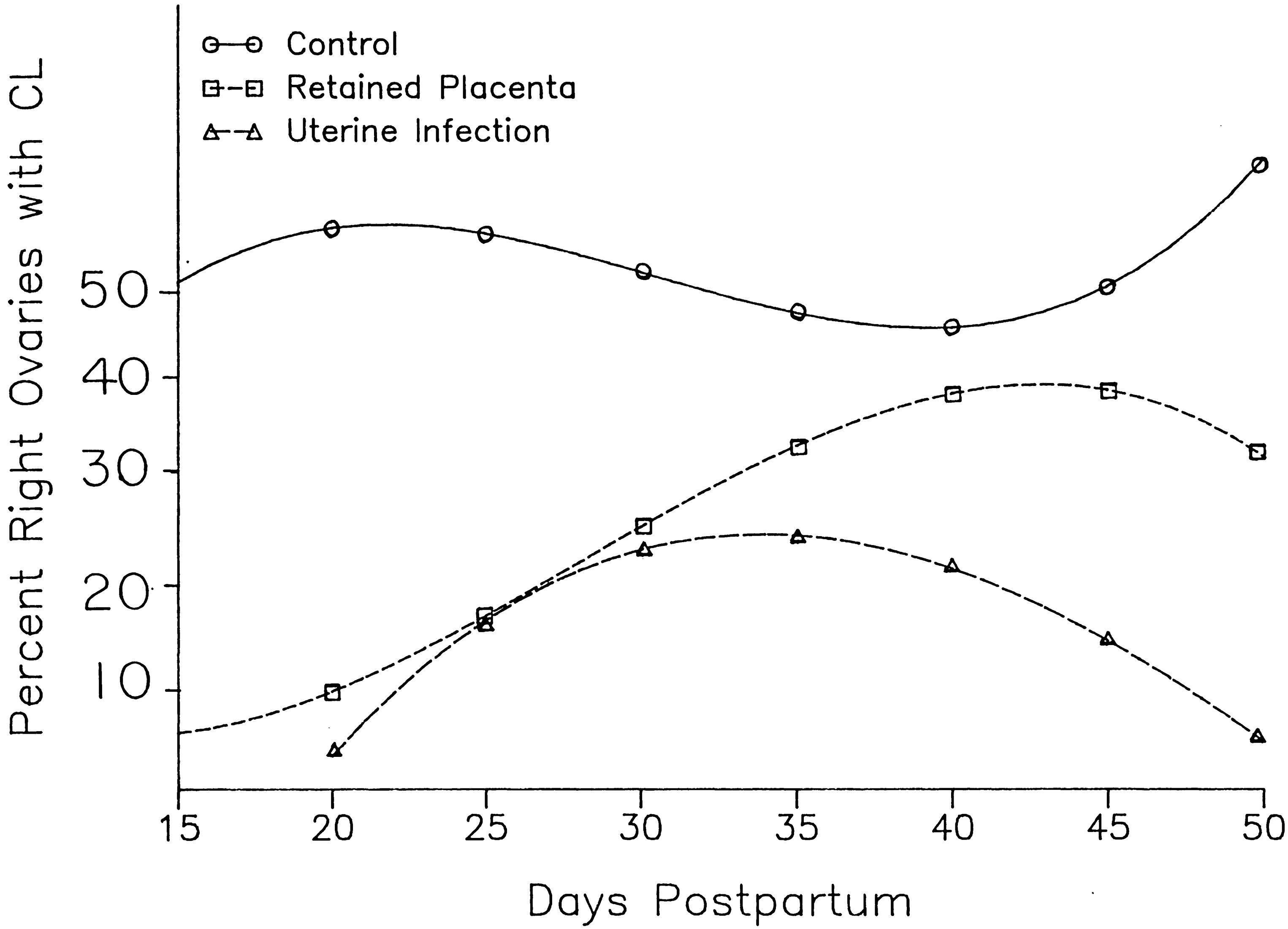
PLASMA PROGESTERONE CONCENTRATION IN COWS WITH RETAINED PLACENTA, UTERINE INFECTIONS AT D 15 OR WERE CONTROL COWS, OVER TIME



Progesterone (ng/ml plasma)
2.5
2.0
1.5
1.0
0.5
0.0
15
20
25
30
35
40
45
50
Days Postpartum
Uterine Infection
Retained Placenta
Control

FIGURE 9

PERCENT RIGHT OVARIES WITH CORPERA LUTEA IN COWS WITH RETAINED PLACENTAS, UTERINE INFECTIONS AT D 15 OR WERE CONTROL COWS, OVER TIME


Control
Retained Placenta
Uterine Infection
Percent Right Ovaries with CL
10
20
30
40
50
15
20
25
30
35
40
45
50
Days Postpartum

luteal phase which is a common occurrence with the presence of a metritic condition in the early postpartum cow (Callahan et al., 1971; Mortimer et al., 1983). Additionally, UI cows had the highest peak progesterone concentrations which were also indicative of a pyometric condition.

Less than 5% of the cows in the UI group had a palpable CL at d 20 postpartum. This increased to slightly greater than 25% at d 35 and decreased to 5% by d 50 postpartum. Therefore, unlike the C group, UI and RP cows had exhibited only one luteal phase over this 30 d period.

Both RP and UI cows had a functional CL as indicated by plasma progesterone concentrations one and three days earlier, respectively, than metritic cows in a study by Callahan et al. (1971) who reported a functional CL at d 30 postpartum. Similarly, the control cows were 4 d earlier to form a functional CL than the 25 d postpartum they reported.

Control cows formed functional CL earlier than the other groups with progesterone concentrations exceeding 1 ng/ml by 21 d postpartum (Fig. 8). This agrees with the findings of Callahan et al. (1971) who reported that cows without postpartum reproductive problems ovulated within approximately 20 d of calving. By 50 d postpartum progesterone concentrations had dropped to 1.25 ng/ml from a peak of 1.8 at d 30. This suggests that the majority of the cows in this group had regressed CL from their previous estrous cycle in a normal period of time.

Approximately 50% of the control cows exhibited a CL on the right ovary at d 15 postpartum (Fig. 9). At d 20 postpartum this increased to 57% and then dropped to 47% by d 40 postpartum. A second increase in frequency of CL occurrence on the right ovary resulted in 54% of the C cows having a palpable CL on d 50 postpartum. Therefore, while not indicated by the plasma progesterone pattern, palpation data revealed C cows had two luteal phases by d 50 postpartum (Fig. 9), compared to only one luteal phase in both the UI and RP groups. Unlike the findings of Callahan et al. (1971) the first cycle postpartum was not shortened in these cows.

There was an increased amount of follicular activity in both RP and C between d 40 and 50 postpartum as is evidenced by decreasing progesterone concentrations (Fig. 8), although this is not evident from a graph of follicles on the left ovary (Fig. 7). An increase in tone of the reproductive tract at ovulation may account for the slight increase in tract diameter seen around 40 to 50 d postpartum (Fig. 3,4,5).

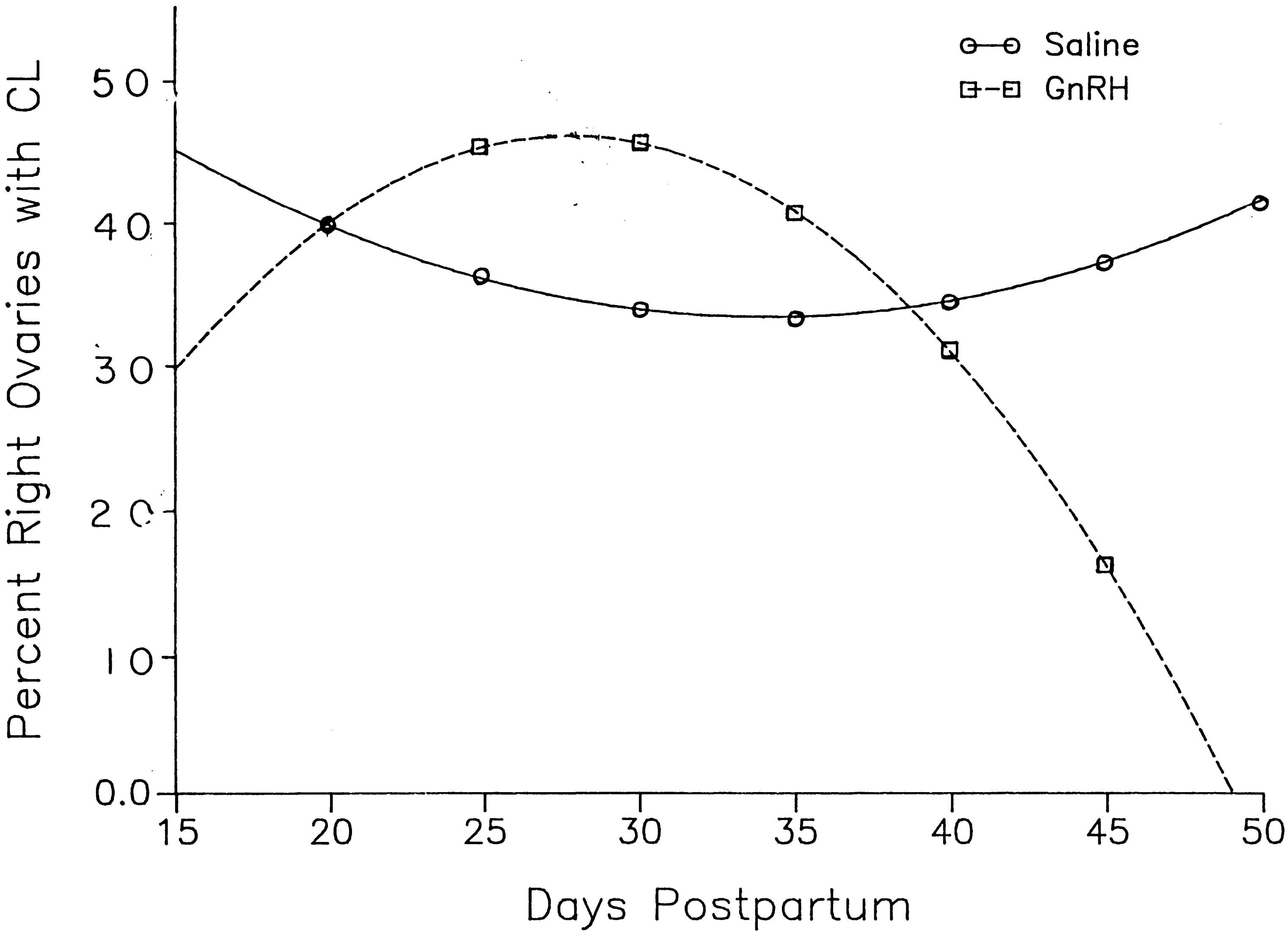
Cows in the UI group had neither an increase in follicular activity as evidenced by the progesterone profile nor an increase in uterine volume. In this group, neither right horn nor cervix diameter increased markedly between d 40 and 50 postpartum (Fig. 6). A very slight increase occurred in left uterine horn diameter from 27 to 28.5 mm (Fig. 4).

Injection of GnRH did not affect progesterone concentrations in this study (Table 7), although it appears that GnRH injection might have led to ovulation (Fig. 7). The progesterone profiles for all three groups indicate low progesterone concentration at d 15 postpartum. Kesler et al. (1978) noted that three of four cows ovulating following GnRH treatment on d 12 to 13 postpartum had a follicle > 1 cm diameter on the day of GnRH treatment. Therefore, follicular maturity is implicated in ovarian response to GnRH injection.

A 10% increase in CL formation on the right ovary was seen between d 15 and 20 postpartum in cows injected with GnRH (Fig. 10). This ac-

FIGURE 10

PERCENT OCCURRENCE OF RIGHT OVARIES WITH CORPERA LUTEA BY TREATMENT GROUP OVER TIME



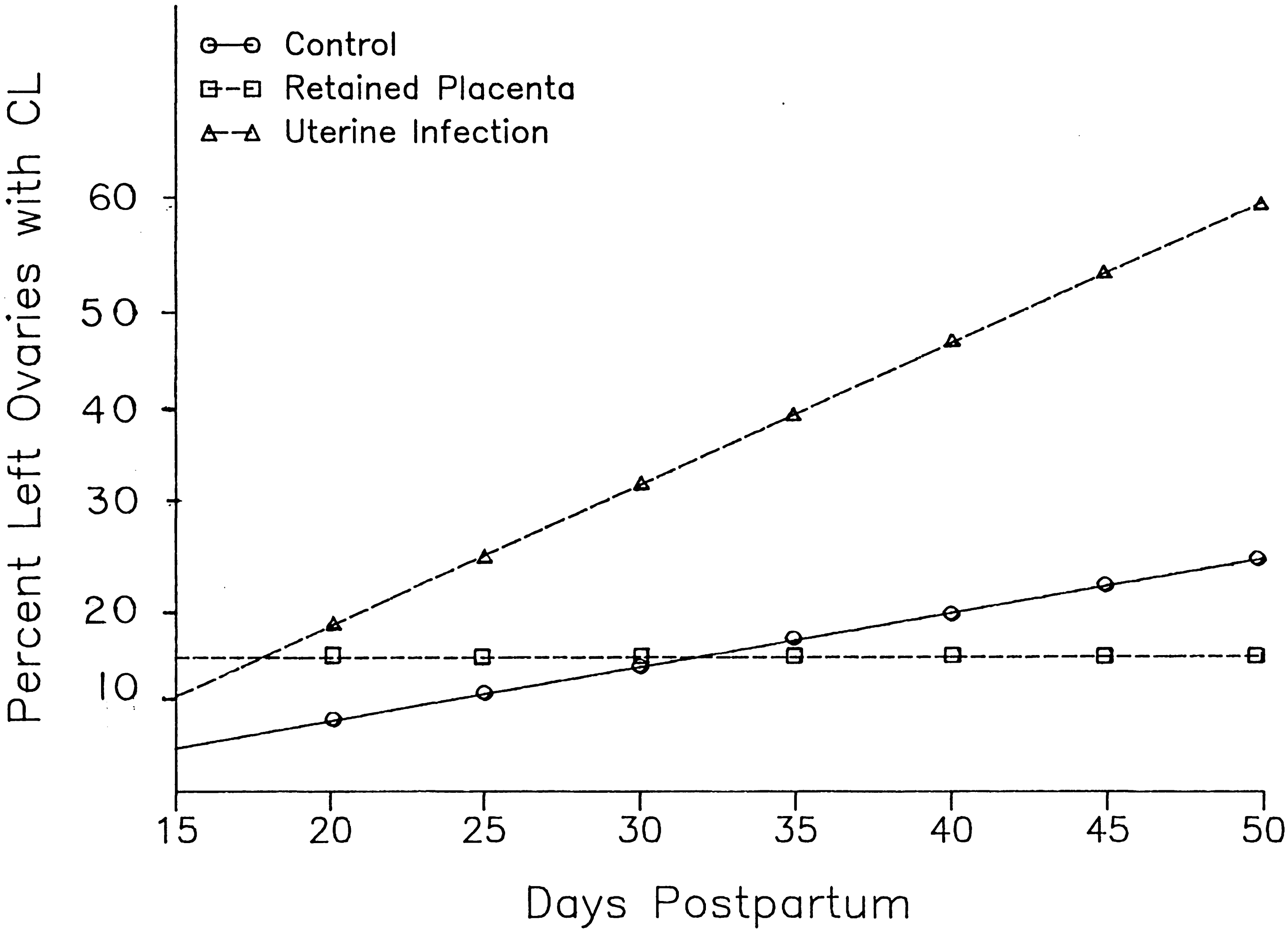
Saline
GnRH
Percent Right Ovaries with CL
50
40
30
20
10
0.0
15
20
25
30
35
40
45
50
Days Postpartum

tivity peaked at 45% between d 25 and 30 postpartum. In contrast, cows injected with saline on d 15 postpartum had a 5% decrease in CL formation on the right ovary between d 15 and 20 postpartum. These findings suggest that GnRH caused ovulation following its injection. However, the lack of difference in progesterone concentration between treatment groups suggests that the CL formed as a result of GnRH injection may not produce as much progesterone as a naturally occurring CL. This phenomenon has been reported previously by Rutter et al. (1986). Kesler et al. (1977) reported that response of a cow to exogenous GnRH in the early postpartum period is governed in part by pre-GnRH injection concentration of plasma LH. Increasing plasma LH concentration prior to GnRH resulted in higher LH peaks following GnRH. Additionally, Callahan et al. (1971) reported plasma LH concentrations were significantly lower in metritic cows at d 8 postpartum than in normal cows. Therefore, it is possible that depressed plasma LH in the RP and UI groups prevented a response of these animals to exogenous GnRH.

The occurrence of CL on the right ovary varied between the clinical groups over time postpartum in a quadratic manner (Table 8). In general, control cows had the highest incidence of CL, 51%. Forty percent of the RP animals had CL on the right ovary between d 15 and 50 postpartum while only 25% of the UI cows had a CL on the right ovary during this time period (Fig. 9). Additionally, occurrence of CL on the left ovary varied linearly between clinical groups over day postpartum (Table 8). On d 15 postpartum only 4.4% of the C cows had CL on the left ovary (Fig. 11). This increased to d 50 at which time there was a 23.7% occurrence. In contrast, the RP

FIGURE 11

PERCENT OCCURRENCE OF LEFT OVARIES WITH CORPERA LUTEA IN COWS WITH RETAINED PLACENTAS, UTERINE INFECTIONS AT D 15 OR WERE CONTROL COWS, OVER TIME



Control
Retained Placenta
Uterine Infection
Percent Left Ovaries with CL
60
50
40
30
20
10
15
20
25
30
35
40
45
50
Days Postpartum

animals showed only a slight increase, having a 13.6% occurrence on d 15 and a 13.8% occurrence on d 50 postpartum.

Sixty-seven percent of the UI cows exhibited a CL on the left ovary at d 50 postpartum compared to a 9.6% occurrence at d 15 (Fig. 11). The high frequency of CL seen at d 50 in this group is supported by the progesterone profile of these animals which indicated elevated progesterone at this time (Fig. 8). As expected, groups with a high occurrence of CL on the left ovary had a low occurrence of CL on the right ovary and vice versa.

A normal luteal phase of 13 d is expected in dairy cows. During the early postpartum period this is often shortened to 7 d. A CL which persists for more than 14 d causes a delay in the occurrence of future luteal phases (Schams et al., 1978). Of cows initially assigned to the UI group, which received GnRH, four of the eleven (36.4%) developed luteal phases in excess of 14 d while only one cow of the eleven (9.1%) in this group which received a saline injection had a luteal phase greater than 14 d.

Prolonged luteal phases were seen in three of ten RP cows which received GnRH and three of ten cows which were injected with saline in the same group. Five C cows (55.9%) injected with GnRH and one (11.1%) injected with saline exhibited prolonged luteal phases. All the control cows which exhibited prolonged luteal phases had vaginal discharge which was clear on d 15 postpartum, but beginning on d 20 and continuing to d 50 they had a discharge containing varying levels of pus. Additionally, small amounts of fluid were palpated in the uterine horns of all these cows. Therefore, although their original classification was as control cows, and they were treated as such for analyses, their actual reproductive profile was most likely more similar to that of animals in the UI group. Therefore, UI and C cows were combined for the purpose of analyzing the frequency of prolonged luteal phases.

Chi-square analysis did not reveal a difference between combined clinical groups and treatment for the frequency of prolonged luteal phase ($P>.05$) (Table 9). However, 56% of all luteal phases in excess of 14 d were observed in UI cows which had been injected with GnRH. Twelve and one-half percent of prolonged luteal phases occurred in RP cows treated with GnRH. Nineteen and 12.5% occurred in saline treated RP or UI cows, respectively.

The number of prolonged luteal phases seen in the UI cows treated with GnRH injection on d 15 postpartum is greater than the 10% observed in metritic cows by Martinez and Thiber (1984). Although, Fonseca et al. (1983) reported that 50% of all prolonged luteal phases occur in cows with uterine infections, the saline treated UI group had a prolonged luteal phase frequency closer to the 10% reported by other researchers. Of all the cows which exhibited prolonged luteal phases, 68.5% had been injected with GnRH and 31.5% received saline. Additionally, 68.5% of all prolonged luteal phases occurred in UI cows and 31.5% in RP cows.

The combination of UI and GnRH appears conducive to the formation of a persistent CL, accounting for nearly 56% of all persistent CL's which occurred (Table 9). In marked contrast, only 2 UI cows treated with saline developed a persistent CL. Therefore, it appears that GnRH was contributing to the formation of a CL with increased persistency. This is further supported by the fact that GnRH treated cows had a lower occurrence of follicles on the left ovary than did saline treated cows (Fig.

Table 9. CHI-SQUARE ANALYSIS OF PROLONGED LUTEAL PHASE BY TREATMENT AND CLINICAL GROUP.[1]

	PROLONGED	NOT PROLONGED
Uterine infection[b] saline treated	12.5(2)[a]	29%(9)
Uterine infection GnRH treated	56%(9)	23%(7)
Retained placenta saline treated	19%(3)	23%(7)
Retained placenta GnRH treated	12.5%(2)	26%(8)

[1]Chi-square=5.7, P>.05, df=3

[a](n)

[b]Uterine infection is comprised of cows from uterine infection and control groups.

7). In contrast, Rutter et al. (1985) reported that CL lifespan following GnRH injection was shortened. The prolonged luteal phases in this study may be a direct effect of pyometra on CL regression.

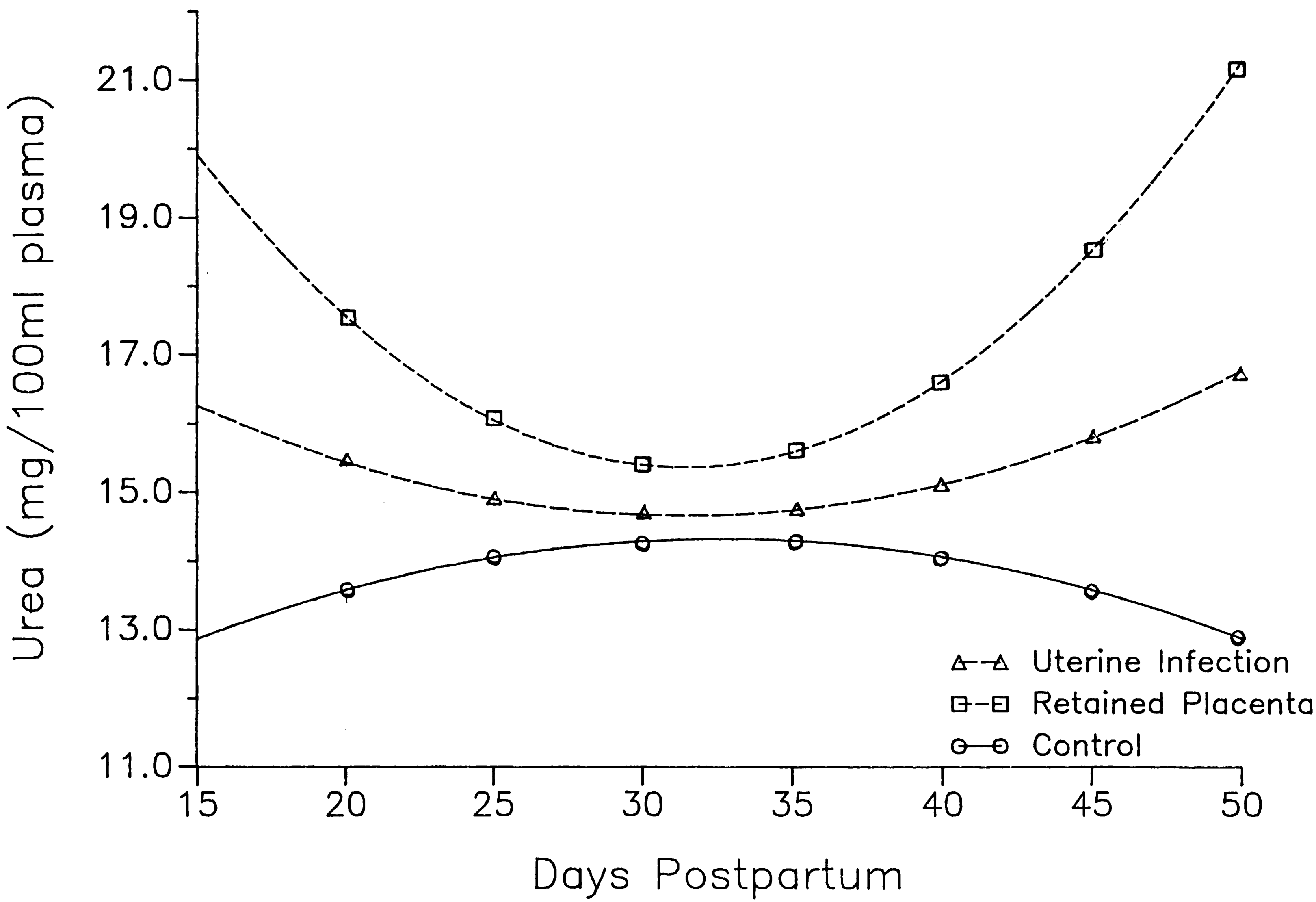
Urea

Urea concentrations between d 15 and 50 postpartum were affected by the treatment by clinical group interaction, and the days postpartum by treatment group and days postpartum by clinical group interaction (Table 7). In all cases plasma urea concentration changed in a quadratic manner over days postpartum (Fig. 12 and 13). Both UI and RP cows had plasma urea concentrations which decreased from d 15 to 30 to 35 d postpartum and then increased to 50 d postpartum. Plasma urea concentration was 20 mg/100ml on d 15 in RP cows and dropped to 16.5 mg/100ml at 30 to 35 d. By 50 d postpartum plasma urea increased to approximately 21.5 mg/100ml. Urea concentration in UI cows was 16.3 mg/100ml on d 15, decreased slightly to 14.7 mg/100ml at d 30, then increased to 16.8 mg/100ml by d 50. Cows without clinical reproductive problems had profoundly different urea profiles than RP or UI cows. Plasma urea concentration averaged 12.2 mg/100ml on d 15 postpartum, increased to 14.3 mg/100ml at 30 to 35 d and then returned to approximately 12.8 mg/100ml by d 50 postpartum.

Plasma urea was also affected by the interaction of treatment group and days postpartum, urea being higher between d 28 and 35 in cows injected with saline than in those treated with GnRH (Fig. 13). Again, plasma urea varied in a quadratic fashion over days postpartum. In a pattern similar to that of the UI and RP cows, plasma urea was high initially in the GnRH treated cows (15.4 mg/100ml) then dropped to a value of 14.4 mg/100ml before increasing slightly to a value of 14.83 mg/100ml.

FIGURE 12

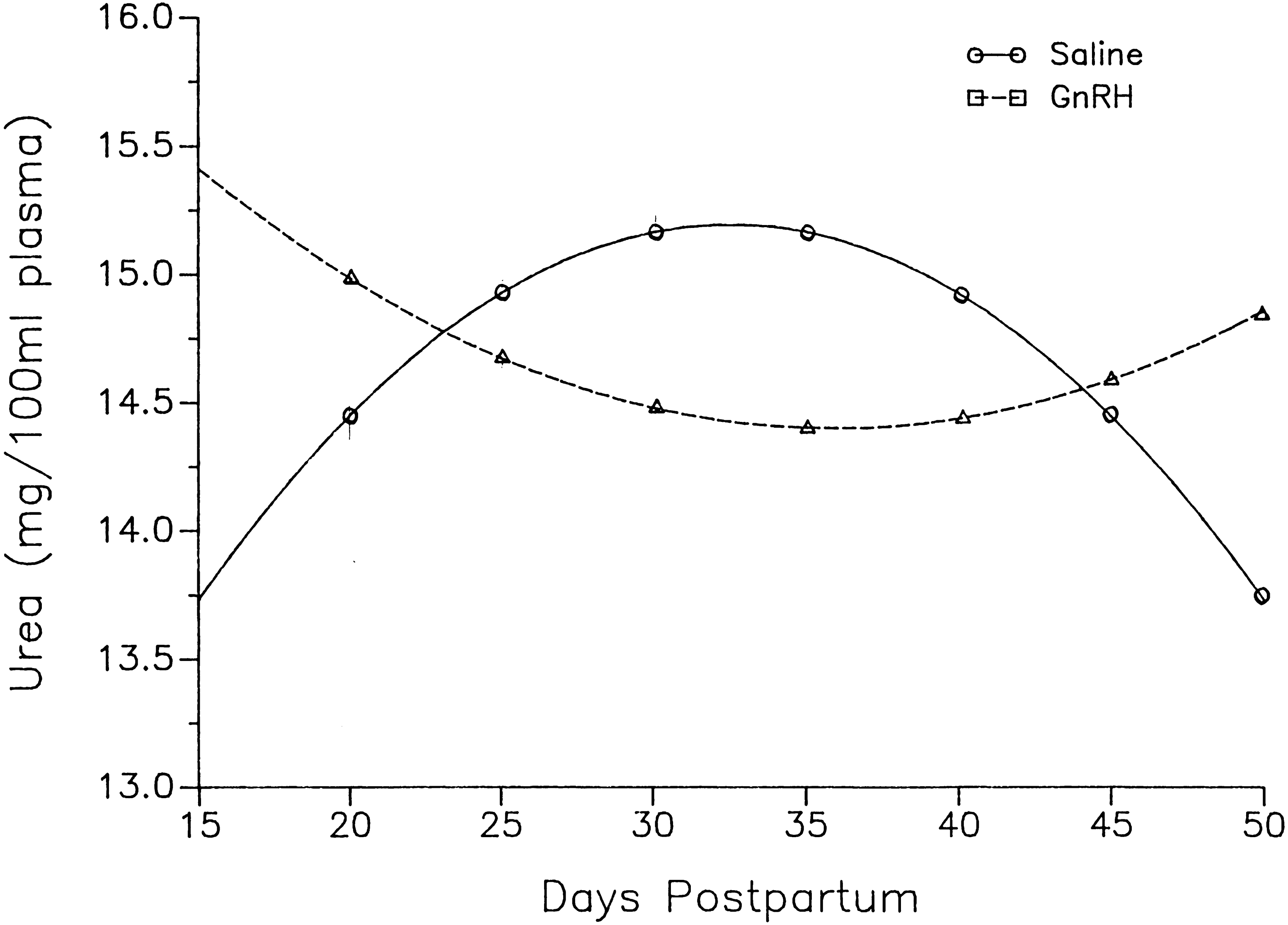
PLASMA UREA CONCENTRATION IN COWS WITH RETAINED PLACENTAS, UTERINE INFECTIONS AT D 15 OR WERE CONTROL COWS, OVER TIME



21.0
19.0
17.0
15.0
13.0
11.0
Urea (mg/100ml plasma)
15
20
25
30
35
40
45
50
Days Postpartum
Uterine Infection
Retained Placenta
Control

FIGURE 13

PLASMA UREA CONCENTRATION BY TREATMENT OVER TIME



Saline
GnRH
Urea (mg/100ml plasma)
16.0
15.5
15.0
14.5
14.0
13.5
13.0
15
20
25
30
35
40
45
50
Days Postpartum

Conversely, saline treated cows had an initial value of 13.75 mg/100ml which then increased to 15.5 mg/100ml and dropped to 13.75 mg/100ml by d 50 postpartum.

The plasma urea concentrations of all the clinical groups at d 30 to 35 is similar the plasma urea reported by Jordan and Swanson (1979) in cows fed high CP diets (16.8 mg/100ml). These plasma urea concentrations were shown to increase uterine urea content. Folman et al. (1973) reported that excess urea decreased plasma progesterone concentrations in dairy cattle, thus causing decreased fertility.

Decreased CL function near d 15 to 20 postpartum as evidenced by low plasma progesterone concentrations in the RP and UI groups may have been mediated in part by elevated plasma urea concentrations. Interestingly, persistent luteal function was more prevalent in the groups of cows which had displayed elevated plasma urea concentrations. Prolonged luteal phases were most prevalent in GnRH treated, UI cows. If LH binding to the CL was inhibited by elevated urea in these groups, the CL would be more susceptible to luteolytic effects of prostaglandins produced by the uterus. Henderson and McNatty (1975) have postulated that the CL becomes more vulnerable to luteolytic prostaglandin effects as LH binding decreases. Because prostaglandins did not seem to be exerting a luteolytic affect on the animals with the lowest theoretical CL binding of LH, it is possible that prolonged luteal activity in this study is indeed a result of altered prostaglandin release due to the metritic state.

Jordan et al. (1983) did not find differences in plasma urea concentrations during the estrous cycle, but did find significant differences between high CP (23 %) diets and low CP (12 %) diets. Several researchers have reported adverse effects of elevated plasma urea on gametes due to increased urea in the uterus (Saitoh and Takahashi, 1977). High amounts of urea in the blood at the time of first service may have altered metabolism of both the sperm and ova , resulting in decreased fertility.

Glucose

Plasma concentrations of glucose were significantly different over time between cows which received GnRH and those which were injected with saline (Table 7). Cows which received GnRH had an average plasma glucose concentration of 63.0 mg/100ml on d 15 postpartum and this declined to 61.9 mg/100ml by d 50 (Fig. 14). On d 15 postpartum plasma glucose concentration was 61.2 mg/100ml in the saline treated cows and increased to 61.8 mg/100ml at d 50.

Hypoglycemia may affect bovine fertility by depressing hypothalamic activity which leads to a subsequent depression of ovarian activity (Oxenreider and Wagner, 1971). As both groups had similar amounts of glucose at the time when insemination could commence, it is unlikely that glucose concentration had any effect on subsequent differences in fertility on the two treatment groups.

Plasma glucose concentration was not significantly different between the three clinical groups (Table 7).

Histology

Heavy occurrence of diffuse lymphocytes was significantly more ($P<.01$) prevalent in RP cows than in C or UI animals (Fig. 15). Most cows did not have diffuse lymphocytes or did so only in moderate amounts. Of the 112 samples containing no diffuse lymphocytes, 39.3% occurred in C

FIGURE 14

PLASMA GLUCOSE CONCENTRATION BY TREATMENT OVER TIME

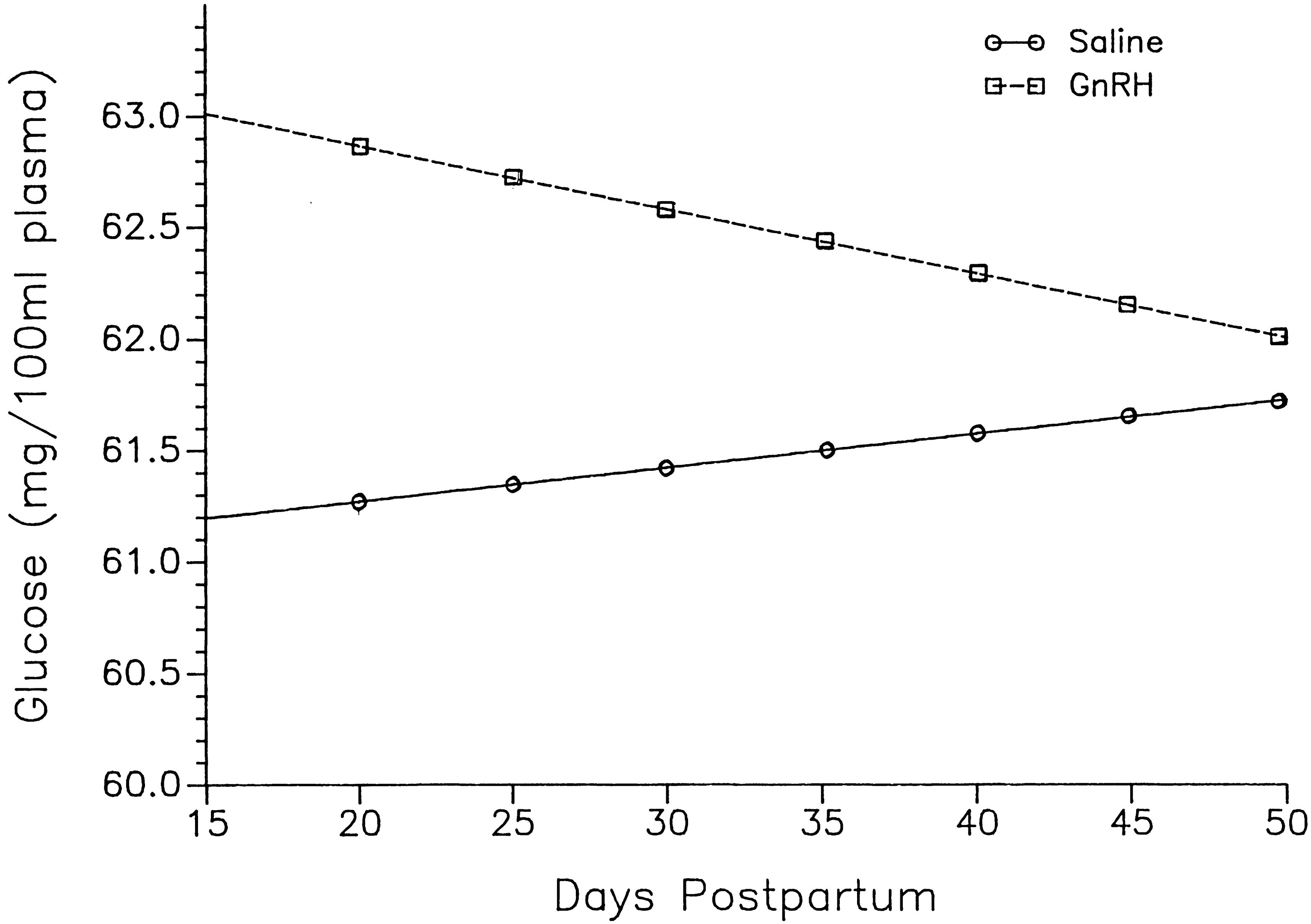
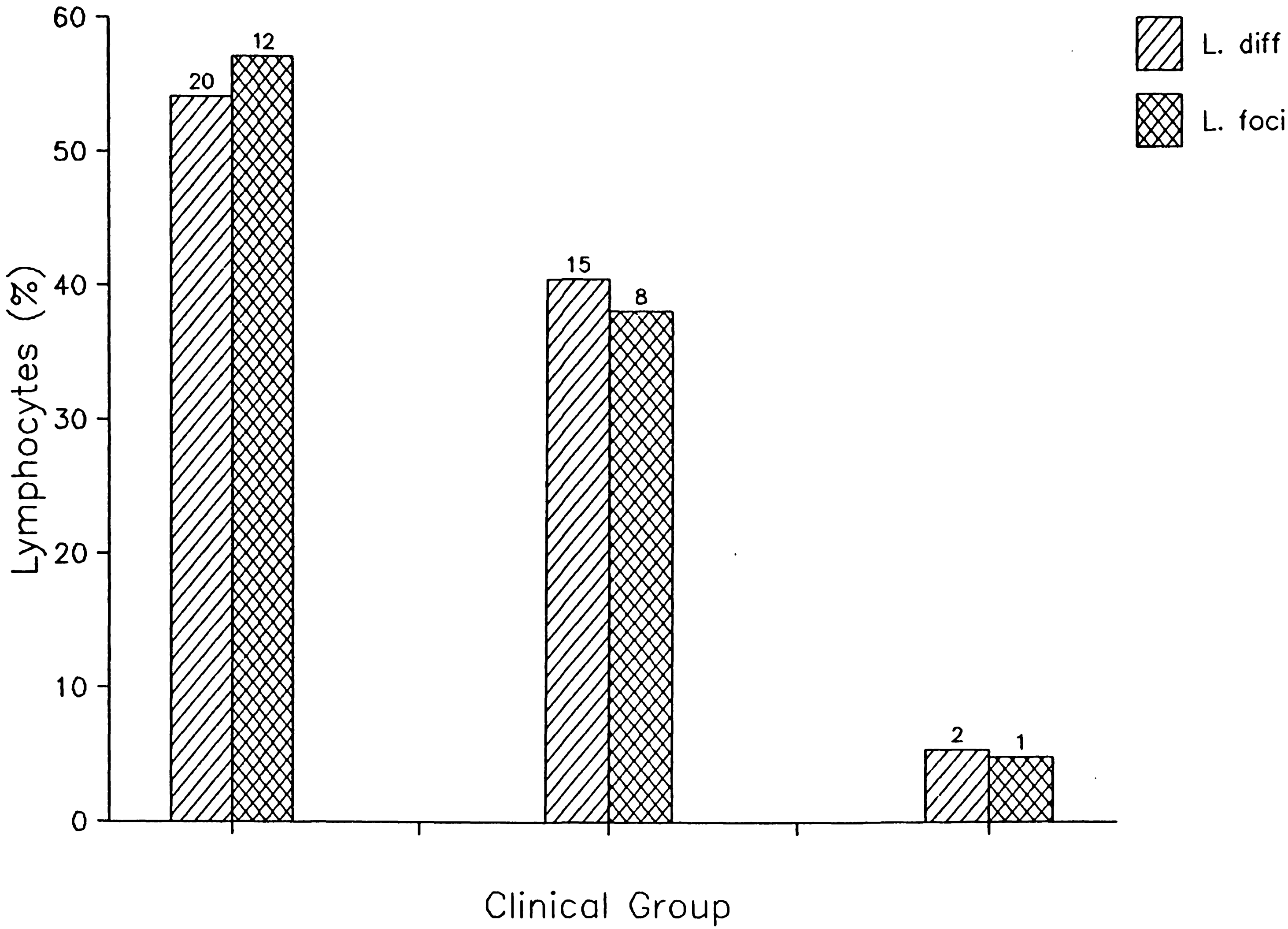

Saline
GnRH
Glucose (mg/100ml plasma)
63.0
62.5
62.0
61.5
61.0
60.5
60.0
15
20
25
30
35
40
45
50
Days Postpartum

FIGURE 15

PERCENTAGE OF UTERINE TISSUE SAMPLES CONTAINING DIFFERENTIAL OR FOCAL LYMPHOCYTES IN COWS WITH RETAINED PLACENTAS, UTERINE INFECTION AT D 15 OR CONTROL COWS (n=ACTUAL OCCURRENCE)


Lymphocytes (%)
60
50
40
30
20
10
0
20
12
15
8
2
1
L. diff
L. foci
Clinical Group

animals and 40.2% occurred in UI cows. Only 20.5% of the samples which did not contain diffuse lymphocytes were obtained from RP cows. This trend was altered in the samples containing moderate amounts of diffuse lymphocytes. Of 98 samples containing moderate amounts of diffuse lymphocytes, 22.5%, 34.7% and 42.9% were from C, UI and RP cows respectively (Table 10). Only 37 samples had a high occurrence of diffuse lymphocytes, over half of these were from RP cows. Fifty-four percent of the 37 samples containing large numbers of differential lymphocytes were from RP cows, 40.5% were from UI cows and 5.4% of the control cows had large amounts of diffuse lymphocytes. The significant negative correlation of diffuse lymphocytes with clinical group ($r=-.26$, $P<.01$) supports the finding that they are much more prevalent in the RP group than in the UI or C groups. The amount of this blood constituent did not change in uterine sample between 2 and 7 wk postpartum (Table 10). Additionally, the occurrence of differential lymphocytes was not different between treatment groups.

The occurrence of fibrosis in uterine samples was not affected by clinical group, treatment group or number of days postpartum (Table 11). However in contrast to Manspeaker et al. (1984) the occurrence of fibrosis was relatively low. These researchers reported that 81% of all cows had fibrosis had during the early postpartum period. In our study 79% of the uterine tissue biopsies collected were free of fibrosis. However, uterine scarring is directly correlated to the presence of C. pyogenes in the uterus (Griffen et al., 1974a).

Neutrophils were more prevalent in biopsies obtained from cows which had been injected with GnRH than those which received saline

Table 10. CHI-SQUARE OF DIFFUSE LYMPHOCYTES BY CLINICAL GROUP AND WEEK POSTPARTUM[a]

	Retained Placenta	Uterine Infection	Control
No occurrence	9%(23)	18%(45)	18%(44)
Moderate occurrence	17%(42)	14%(34)	9%(22)
Heavy occurrence	8%(20)	6%(15)	1%(2)

[a]Chi-square=26, P<.01, df=4.

	WEEK POSTPARTUM				
	2	3	4	5	6
No Occurrence	9.3%(23)	8.9%(22)	10.1%(25)	8.1%(20)	8.9%(22)
Moderate Occurrence	6.9%(17)	8.1%(20)	8.5%(21)	9.7%(24)	6.4%(16)
Heavy Occurrence	3.6%(9)	2.4%(6)	3.6%(9)	2.0%(5)	3.2%(8)

[a]Chi-square=3.6, P>.05, df=8.

Table 11. CHI-SQUARE ANALYSES OF FIBROSIS BY TREATMENT, CLINICAL GROUP AND WEEK POSTPARTUM[a]

	Treatment	
	GnRH	Saline
No occurrence	37% (92)[b]	42% (103)
Moderate or heavy occurrence	13% (31)	8% (21)

[a]Chi-square=2.5, P>.10, df=1
[b](n)

	Clinical Group		
	Retained Placenta	Uterine Infection	Control
No occurrence	28% (69)[b]	31% (77)	20% (49)
Moderate or heavy occurrence	6% (16)	7% (17)	8% (19)

[a]Chi-square=2.7, P<..27,df=2
[b](n)

	Week Postpartum				
	2	3	4	5	6
No occurrence	14%(34)[b]	15%(37)	18%(44)	17%(42)	15%(38)
Moderate or heavy occurrence	6%(15)	4%(11)	4%(11)	3%(7)	3%(7)

[a]Chi-square=4.5,P<.4,df=4
[b](n)

Table 12.CHI-SQUARE ANALYSES FOR OCCURRENCE OF NEUTROPHILS BY TREATMENT, CLINICAL GROUP AND WEEK POSTPARTUM.[a]

	Treatment	
	GnRH	Saline
No occurrence	21%(52)[b]	31%(76)
Moderate occurrence	18%(45)	14%(34)
Heavy occurrence	11%(26)	6%(14)

[a]Chi-square=.6, P<.01, df=2.
[b](n)

	Clinical Group		
	Retained Placenta	Uterine Infection	Control
No occurrence	15%(36)[b]	22%(54)	15%(38)
Moderate occurrence	14%(34)	10%(25)	8%(20)
Heavy occurrence	6%(15)	6%(15)	4%(10)

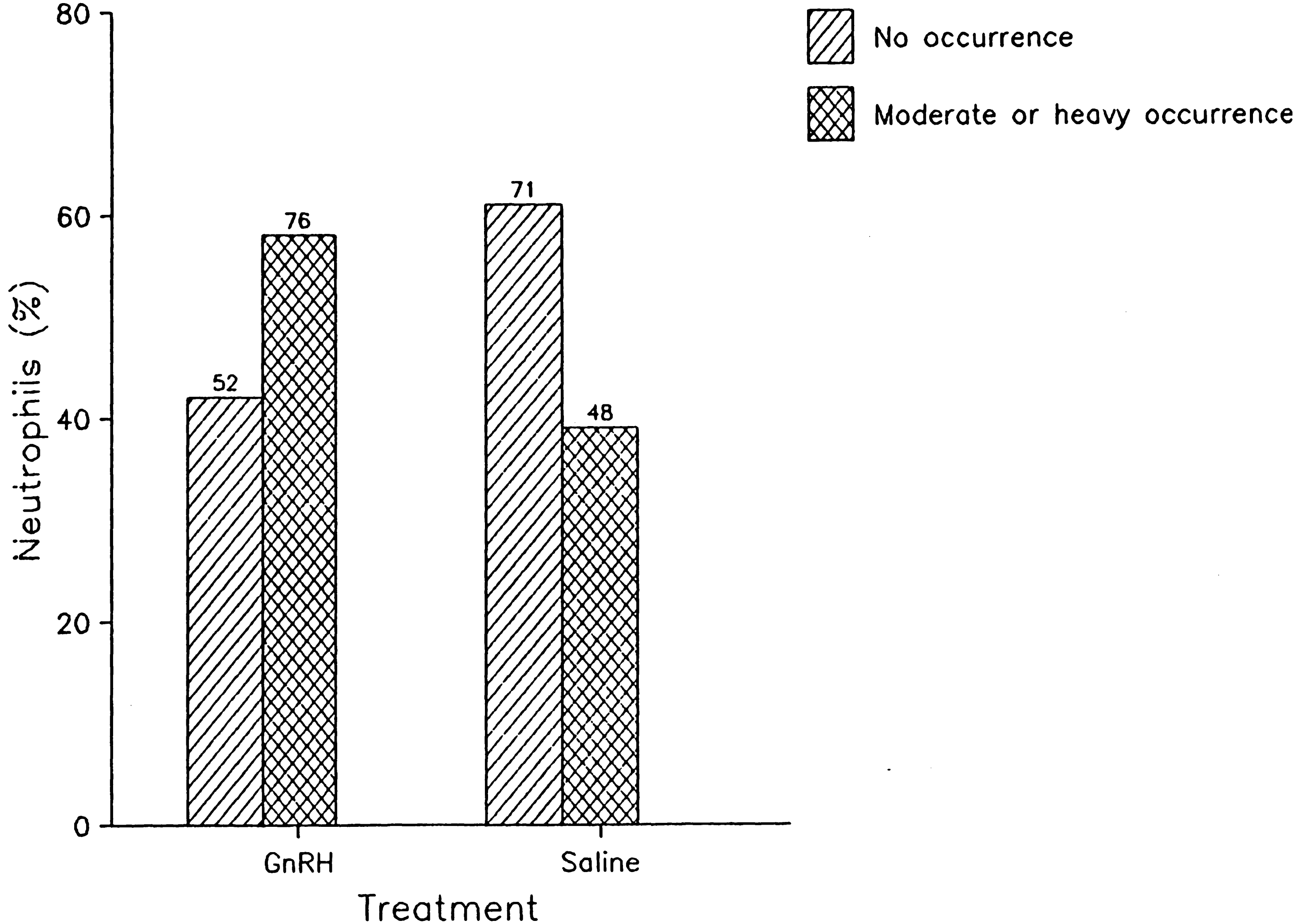
[a]Chi-square=5.1, P<.3, df=4.
[b](n)

	Week Postpartum				
	2	3	4	5	6
No occurrence	6%(14)[b]	8%(19)	11%(27)	13%(33)	14%(35)
Moderate occurrence	7%(17)	8%(21)	9%(21)	5%(12)	3%(8)
Heavy occurrence	7%(18)	3%(8)	3%(7)	2%(4)	1%(3)

[a]Chi-square=38.6, P<.01, df=8.
[b](n)

FIGURE 16

PERCENTAGE OF UTERINE TISSUE SAMPLES CONTAINING NEUTROPHILS BY TREATMENT GROUP (n=ACTUAL OCCURRENCE)



No occurrence
Moderate or heavy occurrence
Neutrophils (%)
0
20
40
60
80
52
76
71
48
GnRH
Saline
Treatment

(Chi-square=9.6, P<.01, df=2; Table 12; Fig. 16). Fifty-seven percent of the samples which exhibited moderate amounts of neutrophils were from GnRH cows as were 65% of the samples with a heavy neutrophil population. Saline injected cows accounted for 59% of the samples which did not contain neutrophils. In all, 58% of the samples from cows injected with GnRH contained neutrophils while only 39% of the samples from saline treated cows contained them.

Focal lymphocytic frequencies were significantly different (P<.05) between the three clinical groups (Fig. 15). Most cows in all groups had no foci at all. Of the 247 specimens which were evaluated, 176, or 71% did not contain foci (Table 13). Twenty percent of the samples had moderate amounts of foci, the fewest samples occurring in RP cows (10) and UI cows having the most frequent occurrences (25). Only 8.5% of all samples exhibited heavy frequencies of focal lymphocytes with 12 occurring in RP cows and 8 and 1 found in the UI and C groups, respectively. Both treatment groups contained similar magnitudes of lymphocytes foci in the uterine endometrium with 29% of the samples from GnRH treated cows containing foci and 28% of the samples from saline treated cows containing foci. Time postpartum, however, did not alter their prevalence (Chi-square=1.2, P>.85, df=4).

Between d 15 and 50 postpartum histological data indicate that uterine inflammation did not change or decreased slightly with the exception of the neutrophilic population (Fig. 17, Table 12).

The number of samples containing neutrophils dropped dramatically from 35 at 2 wk postpartum to 11 at 6 wk postpartum (Table 12). The frequency of diffuse and focal lymphocytes did not show variation over

Table 13. CHI-SQUARE ANALYSES OF FOCAL LYMPHOCYTES BY TREATMENT, CLINICAL GROUP AND WEEK POSTPARTUM.[a]

	Treatment	
	GnRH	Saline
No occurrence	35%(87)[b]	36%(89)
Moderate occurrence	10%(25)	10%(25)
Heavy occurrence	4%(10)	4%(10)

[a]Chi-square=.07, P<1, df=2.
[b](n)

	Clinical Group		
	Retained Placenta	Uterine Infection	Control
No occurrence	26%(63)	25%(61)	21%(52)
Moderate occurrence	4%(10)	10%(25)	6%(15)
Heavy occurrence	5%(12)	3%(8)	.4%(1)

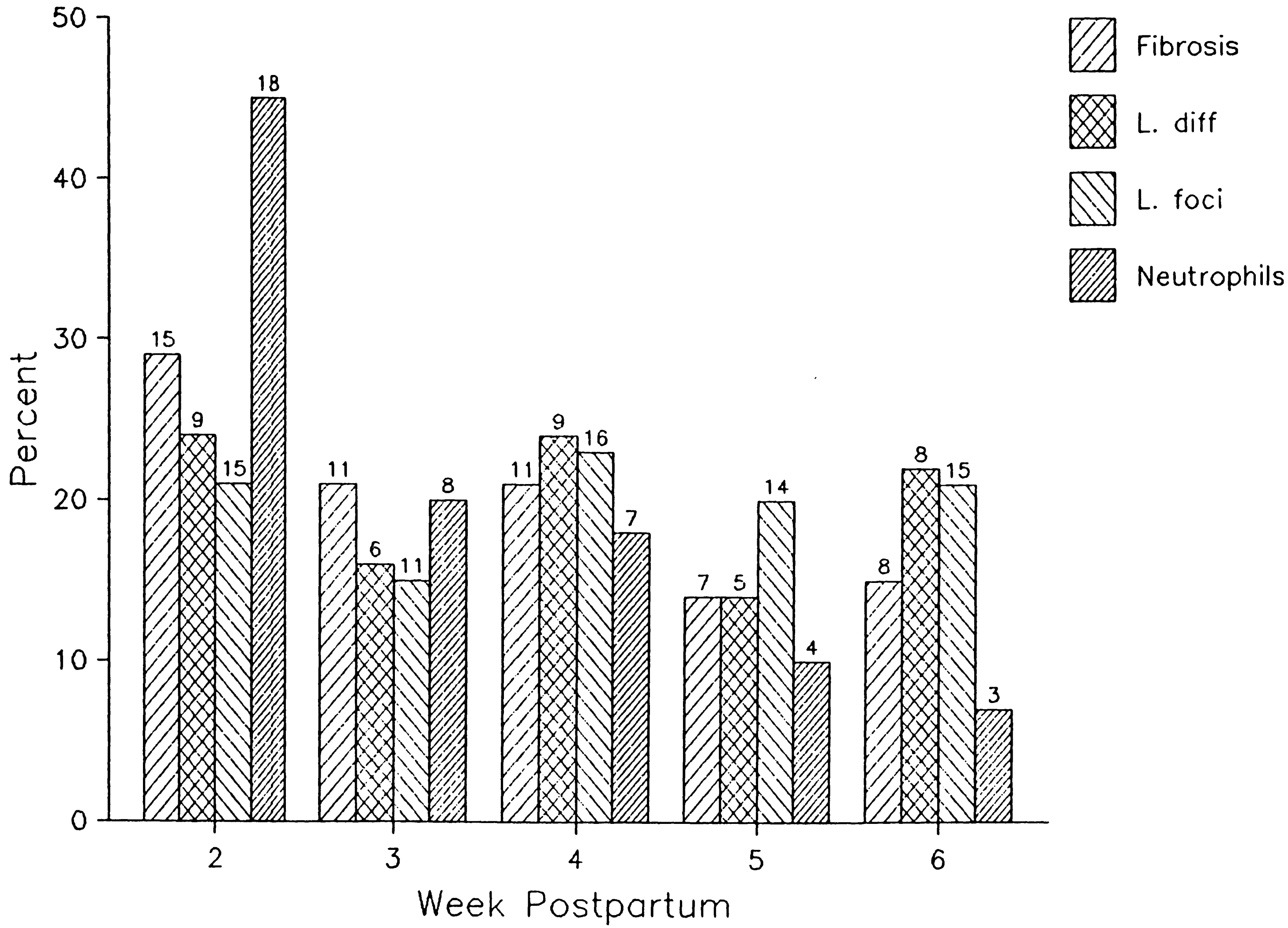
[a]Chi-square=13, P<.05, df=4.

	Week Postpartum				
	2	3	4	5	6
No occurrence	14%(34)	15%(37)	16%(39)	14%(35)	13%(31)
Moderate or heavy occurrence	6%(15)	4%(11)	6%(16)	6%(14)	6%(15)

[a]Chi-square=1.2, P<.9, df=4.

FIGURE 17

PERCENTAGE OF UTERINE BIOPSIES CONTAINING FIBROSIS, NEUTROPHILS, DIFFUSE LYMPHOCYTES (L. DIFF) OR FOCAL LYMPHOCYTES (L. FOCI) OVER TIME (n=ACTUAL OCCURRENCE)



Percent
50
40
30
20
10
0
18
15
9
15
11
6
11
8
11
9
16
7
7
5
14
4
8
8
15
3
2
3
4
5
6
Week Postpartum
Fibrosis
L. diff
L. foci
Neutrophils

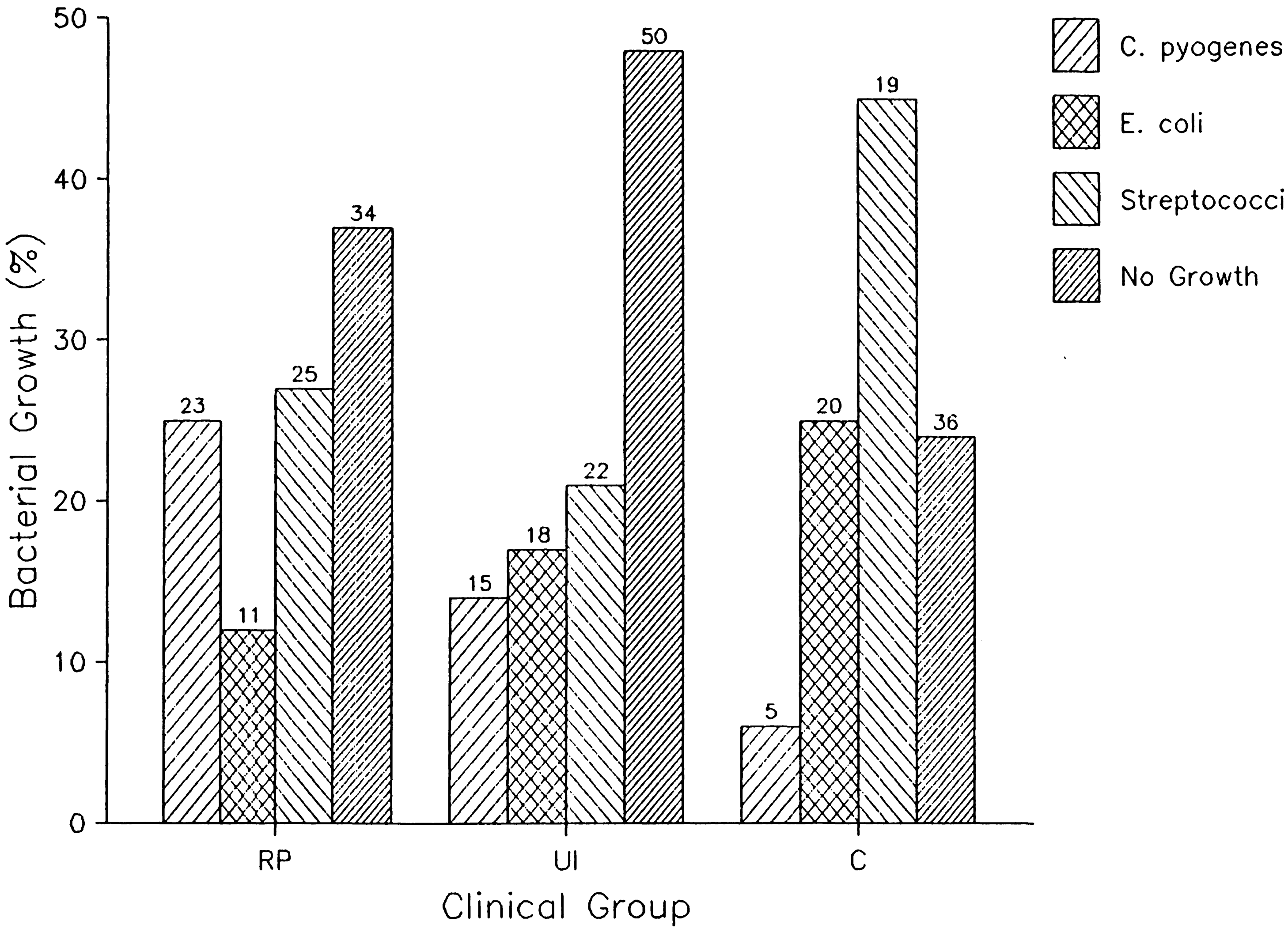
this 5 wk period ranging from 30 to 24 and 16 to 11 occurrences, respectively (Tables 10 and 13, Fig. 17). The occurrence of fibrosis showed a slight decrease, having 15 occurrences in the second wk postpartum and 7 in the sixth wk postpartum (Table 11, Fig. 17).

This is in direct contrast to the findings of Griffen et al. (1974a) who reported a progressive decrease in foci with time postpartum. A possible explanation for this is the markedly higher occurrence of foci by Griffen et al. (1974) than were found in our study. At wk 2 postpartum only 7% of the cows were free of foci (Griffen et al., 1974a) while at the similar time in this study over 69% of the animals did not have endometrial foci (Fig. 8). By wk 7 postpartum this had decreased slightly to 67% which agrees with Griffen et al. (1974a) in which 66% of the samples not containing foci.

It is probable that ovulation and subsequent increased tone of the reproductive tract had a role in occurrence of increased tract diameter between d 40 and 50 postpartum (Fig. 3, 4, 5). This supports the hypothesis that uterine fluid and concurrent increase in lymphocytes and neutropohils did not cause an increase in reproductive tract size. However, Gallagher and Ball (1980) did report an increase in vaginal discharge at the time of ovulation which was not measured in this study. Because the increased tract diameter occurred without a concurrent increase in vaginal discharge score and a very slight change in uterine fluid volume, it seems unlikely that uterine fluid volume was responsible for the increased diameter.

FIGURE 18

PERCENTAGE OF BACTERIA IN UTERINE CULTURES FROM COWS WITH RETAINED PLACENTAS, UTERINE INFECTIONS AT D 15 OR CONTROL COWS, OVER TIME (n=ACTUAL OCCURRENCE)



Bacterial Growth (%)
0
10
20
30
40
50
23
11
25
34
15
18
22
50
5
20
19
36
RP
UI
C
Clinical Group
C. pyogenes
E. coli
Streptococci
No Growth

Bacteriology

Quite often, samples collected from animals with large amounts of vaginal discharge did not result in bacteria being elucidated from culture media (Fig. 18). This is in contrast to Studer and Morrow (1978) who found a positive correlation between discharge and bacterial isolation. Also in contrast with the findings of these researchers, the amount of discharge was not positively correlated to the severity of focal lymphocytes or diffuse lymphocytes although there was a correlation between the presence of C. pyogenes and vaginal discharge score ($r=-.11$; $p<.01$). A negative correlation existed between the occurrence of fibrosis and vaginal discharge score ($r= -.10$, $P<.01$) which indicated an increase in vaginal discharge with an increase in uterine fibrosis. Scarring of uterine endometrium was not affected by bacterial populations nor was it different between clinical groups in this study (Table 11).

Chi-square analysis of bacteria cultured from the three clinical groups indicated that different amounts of C. pyogenes, E. coli and streptocci were found in the groups ($P<.05$) (Fig. 18).

The freuency of C. pyogenes found in uteri of RP cows was higher than that of UI or C cows ($P<.05$). Fifty-three percent of the samples containing this organism were obtained from the RP group, 35% from the UI cows and only 12% from C cows. This is not surprising as θC. pyogenesθ is also associated with vaginal discharge and RP cows had the highest score (Fig. 3). However, C. pyogenes also is related to uterine scarring (Manspeaker et al., 1984) which was not found to vary among clinical groups.

Olsen et al. (1984) have shown CL to be persistent in the presence of C. pyogenes. This is a feasible explanation for the greater incidence of prolonged luteal phases in cows with vaginal discharge and RP cows (Table 9, Fig. 18).

The frequency of occurrence of E. coli was inversely related to the occurrence of C. pyogenes (Fig. 18). Samples from RP, UI and C cows accounted for 22%, 37% and 41% of this organism, respectively. This is most likely explained by E. coli being more prevalent in an environment where it does not have to compete with C. pyogenes. Escherichia coli has not been shown to illicit pathological conditions in the reproductive tract (Studer and Morrow, 1978). It is therefore reasonable that this bacteria is the predominant organism found in animals that do not exhibit clinical reproductive conditions.

Species of streptococci were slightly more prevalent in RP than UI or C cows (38%, 33% and 29%, respectively) (Fig. 18). Like E. coli, this organism naturally occurs in the digestive tract and most was not associated with a pathological condition in the reproductive tract (Studer and Morrow, 1978).

It has been reported (Lindell et al., 1982; Madej et al., 1984) that the period of elevated plasma prostaglandins that occurs immediately following calving is negatively correlated to reproductive tract involution rate and ovulation. Therefore, animals that have low plasma prostaglandin concentrations following calving have a longer interval to involution and ovulation than animals with high plasma prostaglandin concentrations. In this study, involution rate was similar between groups, while ovulation occurred sooner in control cows. This suggests that the clinical conditions were affecting ovarian activity by a mechanism other than reproductive tract involution. Chi-square analysis of bacteria type cultured from the uterus showed significant differences between the two treatment groups ($P<.06$) and the trends evident in this analysis may help to explain the differences in neutrophils found between the treatment groups (Fig. 16). Corynebacterium pyogenes was more evident in cows which received GnRH than those injected with saline (67.4 vs 32.6%). The correlation between C. pyogenes and treatment is significant ($r=.10$, $P<.01$) indicating that indeed, more C. pyogenes was found in GnRH cows. This most likely explains the increased occurrence of neutrophils in this group. Studer and Morrow (1978) indicated that high amounts of neutrophils present in the endometrium were indicative of pathogenic conditions.

Uterine samples which did not produce bacterial colonies when cultured accounted for 43% of all the samples collected. This is a similar occurrence to the 38% reported by Sagartz and Hardenbrook (1971). Of all samples from UI cows, 48% did not yield growth, while 37% of all samples from the RP group responded similarly to culturing (Fig. 18). A combined total of 42% of samples from cows with clinical problems did not result in bacterial growth. This is in contrast to Sagartz and Hardenbrook (1971) who recovered only 34% no growth swabs from cows with clinical evidence of metritis. A large number of these "no growth" swabs were obtained from animals which exhibited large amounts of discharge. As the correlations between vaginal discharge and biopsy results of this study agree with the findings of other researchers, it is difficult to explain the discrepancy between vaginal discharge and lack of bacterial growth. It is possible that the discharge was due to anaerobic bacteria rather than the aerobes whose growth was being monitored. However, the only anaerobic bacteria found to be of consequence in bovine uterine infection are F. necrophorum and B. melaninogenicus. These pathogens are strongly correlated to the presence of C. pyogenes. Therefore, if these bacteria were responsible for the discharge, it seems that C. pyogenes would have been present with greater frequency.

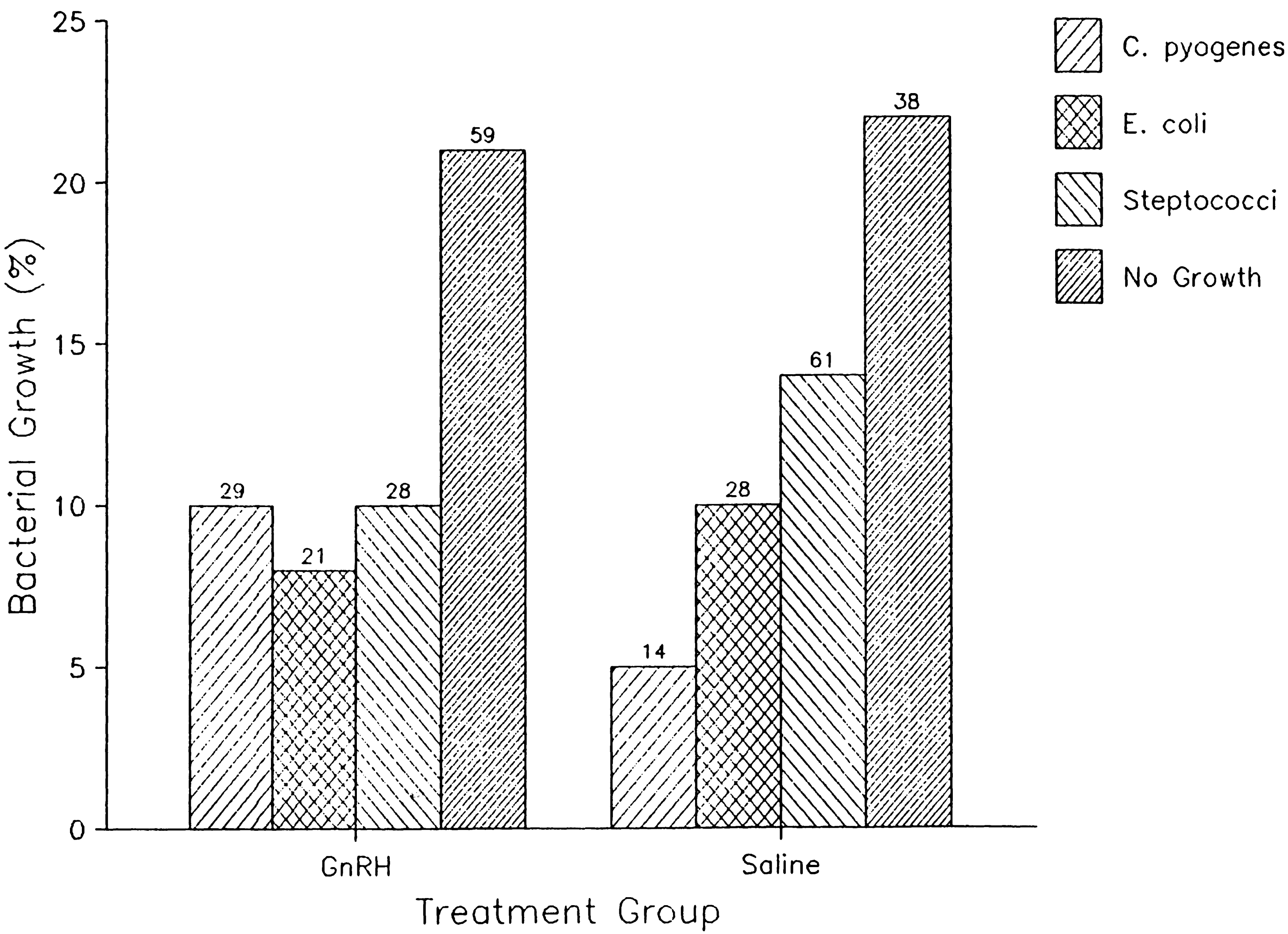
Of the 158 samples which resulted in bacterial growth, 115 contained the non-pathological bacteria E. coli and streptococci species. Only 43 of a total of 278 samples, or 15%, contained C. pyogenes. This is lower than the 22% occurrence reported by Hartigan et al. (1974). It is possible that antibacterial blood components had already killed bacterial cells which were obtained on the uterine swabs. Polymorphonuclear neutrophils have been shown to be present in similar concentrations in bovine plasma and uterine flushings following induced uterine infections (Anderson et al., 1985). These same antimicrobial agents would be responsible for large volumes of vaginal discharge.

The types of bacteria cultured from the two treatment groups were significantly different ($P<.06$) (Fig. 19). C. pyogenes was twice as prevalent in the cows which received GnRH as compared to those cows injected with saline (67% versus 30%) . As stated earlier, this difference is supported by the greater occurrence of neutrophils obtained from tissue samples of GnRH treated cows.

Unlike the findings of Hartigan et al. (1974) no change occurred in the frequency of bacterial growth over time (Table 14). However, affects of time postpartum may have been masked by the large percentage of swabs which did not result in bacterial growth.

FIGURE 19

OCCURRENCE OF BACTERIA CULTURED FROM UTERI OF COWS BY TREATMENT GROUP (n=ACTUAL OCCURRENCE)


Bacterial Growth (%)
0
5
10
15
20
25
29
21
28
59
14
28
61
38
GnRH
Saline
Treatment Group
C. pyogenes
E. coli
Steptococci
No Growth

However, the pattern of uterine bacteria cultured was different over time postpartum. During the second wk postpartum 26% of all samples resulted in growth of C. pyogenes, 20% in growth of E. coli, 7% in growth of streptococcus species and 35% did not yield bacterial growth. By 6 wk postpartum only 9% of the samples contained C. pyogenes, 20% contained E. coli, 20% resulted in growth of streptococcus colonies and 24% did not yield bacterial growth (Table 14). This shows a decrease in the pathogenic C. pyogenes while less detrimental bacteria increased in number.

Reproductive Efficiency

The number of days from calving to conception was affected by clinical group (P<.09), but not by GnRH injection (Table 15). The days open ranged from 134.3 for RP animals to 96.9 for C cows. Animals in the UI group were intermediate with an average of 117.9 days open (Table 16). This is in agreement with the findings of Studer and Morrow (1978) in which gross genital tract condition affected days open. Martinez and Thiber (1984) also reported an increase in days open with the occurrence of uterine infections in the early postpartum period. However, unlike these researchers, vagianl discharge score was not related to days open. In contrast to Fonseca et al. (1983) neither age nor milk yield during the first 100 d postpartum affected this reproductive variable (Table 15).

Services per conception followed a trend among clinical groups similar to the pattern of days open. Cows in the RP, UI and C groups required 2.5, 1.7 and 1.6 services per conception, respectively (Table 16). This significant difference of services per conception between clinical groups (P<.07) is similar to that reported by Studer and Morrow (1978). However,

Table 14. CHI-SQUARE ANALYSIS OF BACTERIA BY WEEK POSTPARTUM[a].

	Week Postpartum				
	2	3	4	5	6
C. pyogenes	5%(14)	4%(12)	1%(4)	3%(8)	2%(5)
E. coli	4%(11)	3%(9)	4%(11)	3%(8)	4%(11)
Streptococci	3%(9)	4%(11)	5%(15)	6%(17)	5%(13)
No growth	7%(19)	8%(22)	10%(29)	9%(25)	9%(25)

[a]Chi-square=14, P<.3, df=12.

Table 15. ANALYSES OF VARIANCE FOR SERVICES PER CONCEPTION (SC), CONCEPTION RATE (CR), AND DAYS OPEN (DO) FOR CLINICAL GROUP (CG), TREATMENT GROUP (T), MILK YIELD AND AGE.

	SC		DO		CR	
Source	df	MS	df	MS	df	MS
T	1	1.4	1	284	1	.113
CG	2	4.0*	2	5975**	2	.204
T X CG	2	.5	2	688	2	.328
Milk Yield	1	1.7	1	191	1	.002
Age	1	1.9	1	3819	1	---
Error	43	1.2	51	2387	118	.247

* P<.07

** P<.09

Table 16. LEAST SQUARES MEANS ± STANDARD ERROR FOR FERTILITY VARIABLES AND MILK YIELD BY CLINICAL GROUP.

Variable	Retained Placenta	Uterine Infection	Control
Services/conception	2.5±.3[a]*	1.7±.3[b]	1.6±.3[b]
Conception rate (%)	34.8±.07	48.0±.08	44.4±1.0
Days open	134.3±11.0[a]	117.9±10.7[a,c]	96.9±12.4[b,c]
Milk yield(kg/100d)	3102.0±55.9	2645.5±53.3	3247.4±60.7

*Different superscripts are different across rows. (P<.07).

Dohoo (1983) found that services per conception were similar for cows with or without uterine infections and cows with other forms of health problems soon after parturition. Cows with uterine infections did not require significantly more services per conception than did C cows, but retained fetal membranes appeared to have an adverse effect on fertility. As RP cows had the highest incidence of C. pyogenes it is likely that this pathogen contributes significantly to decreased fertility. Other researchers have related fibrosis to both C. pyogenes and increased services per conception (Hartigan et al., 1974; Manspeaker et al., 1984). However, the occurrence of fibrosis was not significantly different between groups (Table 11). Control cows exhibited the greatest frequency of uterine scarring. Thirty-six percent of all the samples exhibiting scarring were obtained from C cows while 33 and 31%, were obtained from UI and RP cows, respectively.

All cows were inseminated at the first signs of estrus occurring after 45 d postpartum. Days to first breeding was not effected by treatment group or clinical group. Control cows were first bred an average of 72±7.0 d postpartum, UI and RP cows were first bred at 85±8.9 and 87±8.8 d postpartum, respectively. Control cows had 21 fewer days open than the UI group, but similar services per conception. This suggests that the UI cows were later to first estrus. The prolonged luteal phase observed in a number of the UI cows may account for the delayed time to first breeding. This is supported by the elevated progesterone concentrations still observed in UI cows at d 50 postpartum (Fig. 8). The other groups had low progesterone concentrations at this time, indicative of imminent ovulation. As the majority of the cows which exhibited prolonged luteal phases had received GnRH it is probable that this exogenously administered hormone may actually be detrimental to the reproductive efficiency of cows with clinical reproductive conditions in the early postpartum period.

The average of days open were almost identical between the two treatment groups, being 114±9.3 for GnRH injected cows and 118±9.0 for cows receiving saline injections. An average of 1.64±.2 services per conception was required for cows injected with GnRH and 1.76±.2 services per conception were required for saline injected cows. This again suggests that GnRH did not have an impact on fertility in this study.

Services per conception were not affected by 100 d milk yield or age of the cow (Table 15). Cows in the RP group produced 29.3 kg/d during the first 100 d of lactation while cows in the UI and C groups produced 29.1 and 33.1 kg/d, respectively. Fonseca et al., (1983) reported a slight antagonism between milk yield and days open in Jerseys. This was in contrast to the present study in which the C cows had the highest 100 d milk yield as well as the fewest services per conception and fewest days open. It is possible that abnomal uterine health and elevated plasma urea in the RP and UI cows as compared to the C cows offset any advantage of slightly lower milk production in these groups.

Conception rate to first service did not vary between the treatment groups, clinical groups or with varying milk yield. The lowest conception rate of 34.8% was seen in the RP group. The UI cows showed the highest conception rate of 48.0%, while C cows had an intermediate first service conception rate of 44.4%. Thirty-nine percent of all first inseminations were fertile in GnRH cows while saline treated cows had a first service conception rate of 45.6%. In contrast, Studer and Morrow (1978) found a negative correlation between milk yield and conception, although conception rate was not affected by gross genital condition.

Age of the cow and calf weight affected which clinical group a cow entered ($p<.05$) (Table 17). Cows were classified as being in their first, second, third or fourth and greater lactation. Cows in their second lactation had a greater incidence of RP and UI (Table 6). The fewest reproductive problems occurred with third lactation and older cows. This is in contrast to Muller and Owens (1974) who reported an increase in the incidence of RP with increasing age.

Cows which produced large calves were more likely to encounter postpartum reproductive abnormalities than cows which produced lighter calves. An average calf weight of 46.7±1.7 kg was recorded for cows with RP compared to 44.8±1.0 kg for UI and 43.7±.9 kg for C cows. A minimum calf weight of 36.4 to 36.8 kg was recorded in all three groups with a maximum weight of 75.0 (twins), 59.1 and 50.0 kg in RP, UI and C groups, respectively. Only one set of twins occurred in this study. Similarly, Muller and Owens (1974) reported heavier calf weights in cows which had RP than in normally calving cows.

There was no effect of season on the subsequent reproductive health of cows in this study (Table 17). Seasons were divided with mid-September to mid-November and mid-March to May being grouped as one season. Mid-November to mid-March and May to mid-September were considered separately. All three seasons had similar numbers of cows from each clinical group. Muller and Owens (1974) reported a higher incidence of RP from August through November.

Table 17. ANALYSIS OF VARIANCE FOR CLINICAL GROUP ASSIGNMENT BASED ON AGE, CALF WEIGHT (CW), CALF SEX (CS) AND SEASON OF CALVING.

Source	df	MS
Age	3	2.29**
CW	1	2.34*
CS	2	.11
Season	2	.13
Error	41	.52

* P<.05
** P<.01

CHAPTER V

SUMMARY

Assignment of cows to clinical groups based on retention of fetal membranes for 24 h and nature of uterine discharge at 15 d postpartum resulted in 3 distinctly different groups of cows. This procedure of assigning groups can be used with confidence in the future.

Involution of the reproductive tract was not affected by either the cows clinical condition or the injection of GnRH. This is possibly due to C cows being older than UI or RP cows and therefore having larger tracts due to increased age.

The nature of vaginal discharge was distinctly different between the three clinical groups with UI cows having the most purulent discharge between 2 and 6 wk postpartum. These cows also showed the most improvement in uterine discharge over the time period studied. Cows in the RP group had discharge which was slightly less purulent than that of UI cows. However, these animals showed little difference in nature of uterine discharge. Control cows had the least purulent discharge of the three groups and also showed little difference in nature of the discharge between d 15 and 50 postpartum.

The frequency of follicles on the left ovary and CL on the right ovary was affected by injection of GnRH. Follicular frequency decreased over time postpartum in both treatment groups, however, saline treated cows had a higher frequency at 15 d postpartum than did GnRH treated cows.

The occurrence of right ovary CL was fairly consistent over time postpartum in the saline treated group. Cows injected with GnRH showed an initial increase in CL occurrence from d 15 to 25 postpartum and then a sharp decline from d 40 to 50 postpartum.

The frequency of CL on the right ovary was also affected by the cows clinical group. Most right ovary CL occurred in the C group and the least occurred in the UI group.

Similarly, plasma progesterone concentrations were different between the clinical groups over time. Control cows reached plasma progesterone concentrations of greater than 1 ng/ml by d 21 postpartum, UI cows by d 27 postpartum and RP cows by d 29 postpartum. Based on plasma progesterone profiles and palpation data, the highest occurrence of prolonged luteal phases occurred in UI cows injected with GnRH.

Plasma urea concentration was affected by both treatment and clinical groups. Plasma urea was higher in cows in saline injected cows than GnRH treated cows. Additionally, cows in the UI and RP groups had higher plasma urea than C cows.

Plasma glucose concentration over days postpartum was significantly different between the two treatment groups. Although GnRH treated cows had lower plasma glucose concentrations at d 15 postpartum than did saline treated cows, there was little difference at d 50 postpartum. Therefore, plasma glucose probably did not affect reproduction in this study.

Histological evaluation of uterine tissue biopsies revealed higher occurrences of diffuse and focal lymphocytes in the UI and RP groups than in the C cows. Neutrophils were more prevalent in cows injected with GnRH and fibrosis was not affected by either GnRH or clinical group. Additionally, the neutrophil population decreased with time postpartum while the other blood components showed little change. This suggests that indicators of uterine inflammation changed little between 2 and 6 wk postpartum.

Bacterial culture of the uterus indicated that most C. pyogenes was cultured from RP cows, most E. coli from C cows and most streptococci from RP cows. More C. pyogenes was cultured from GnRH than saline treated cows and most likely explains the higher incidence of neutrophils in this group. Bacterial growth did not change significantly with time postpartum.

In conclusion, control cows had the fewest days open and the fewest services per conception in this study. This is most likely due to a combination of earlier increase in plasma progesterone, lower plasma urea, less uterine inflammation and fewer uterine pathogens than the UI or RP cows.

Injection of GnRH did not affect reproductive efficiency in this study. Any beneficial effects of GnRH on these cows was most likely obliterated by increased plasma urea, increased neutrophils and increased occurrence of C. pyogenes in these cows. Additionally, cows injected with GnRH had a higher occurrence of prolonged luteal phases than cows injected with saline.

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