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# PROSTAGLANDIN METABOLITE IN BLOOD AND MILK OF WATER BUFFALOES: EVALUATION OF A POSSIBLE INDICATOR OF UTERINE INFECTION

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## EXECUTIVE SUMMARY:

Uterine infections in cattle and buffaloes are common during the first 50 to 60 days after calving. The consequences of uterine infections range from no effect on any measure of productivity, to permanent sterility, to death of the cow. Fortunately, few cows die from uterine infections, but the infections are consistently associated with reduced fertility and reduced overall reproductive performance. In addition, uterine infections often reduce milk yield and usually reduce the profit potential of a cow. Early diagnosis and proper management of uterine infections may reduce the impact of the disease. Therefore, the major purpose of this project was to develop a simple, reliable, environmentally-safe method for improving the diagnosis of uterine infections in cows. Cows with uterine infections often do not have any obvious clinical signs of a problem, because the infection is confined to the uterus. So, a "blood test" may improve diagnosis. After suspect cows are identified with the "blood test", they can be examined thoroughly for clinical signs of the disease and managed accordingly.

During this project, we developed a new method (enzymeimmunoassay; EIA) for measuring a compound (13,14-dihydro-15-keto-$PGF_{2\alpha}$; usually called PGFM) in blood. We used the EIA to determine experimentally whether PGFM values could be used to identify cows with uterine infections. Based on our experiments, PGFM increased in cows with the most common type of uterine infection.

The results from this project indicate that our EIA for PGFM can be used to improve the diagnosis of uterine infections. This allows us to identify cows that need special attention and manage them according. Our "blood test" is simple, environmentally safe, and ideally suited for use in lesser developed countries (LDC). Indeed, we were able to successfully transfer the EIA technology to Honduras and establish the first hormone laboratory in Honduras for the study of reproductive problems in farm animals. In addition, this project gave scientists the opportunity to conduct modern reproductive endocrinology experiments with farm animals in Honduras. Because several people were trained to conduct the EIA and participate in the experiment with cattle in Honduras, this project enhanced the research capabilities of Honduras. It also has improved the teaching program at the Escuela Agricola Panamericana (Zamorano); students were involved in almost every aspect of the project, so they became keenly aware of the usefulness of various assay technologies.

In summary, this project allowed us to develop a simple and safe method for improving the diagnosis of a certain type of uterine infection in cattle. It also improved the research and teaching capabilities of animal scientists in Honduras. Because of the mission of the Escuela Agricola Panamericana (i.e., to involve students in all aspects of agriculture, including research), the impact of this project will be multiplied several fold. That is, this project has given scientists at the Escuela Agricola Panamericana the opportunity to conduct research on the reproductive endocrinology of farm animals and show their students how modern reproductive technologies can be used to improve livestock management. Those students will carry that knowledge to several Latin-American countries.

**NOTE:** Originally, this project was designed to address a problem in water buffaloes in Pakistan. The government of Pakistan was unable to comply with the terms of the subcontract for the project. At the same time, the Pakistani government was unable to convince the U.S. government that they were not involved in producing nuclear weapons, which prevented them from receiving various types of foreign aid. So after long delays, the LDC-portion of the project was shifted to Honduras.

## RESEARCH OBJECTIVES:

Uterine disorders, primarily nonspecific uterine infections, reduce the reproductive efficiency of cows. In some herds, 40% of the postpartum cows may be diagnosed with, and treated for, uterine infections. In addition to reducing reproductive efficiency, uterine infections usually increase herd health costs, and they often reduce feed consumption, cause an appreciable reduction in milk yield, and force producers to cull cows that would otherwise be productive and remain in the herd. Clearly, uterine infections can have a major impact on the profitability of a dairy operation (see manuscript by G. S. Lewis in Appendix; the manuscript is an invited review article that will be published in the Journal of Dairy Science in late 1995 or early 1996).

The preceding statements are based on data from studies in developed countries. Data, albeit limited, from lesser developed countries indicate that the problem is more common and usually more severe and the consequences are greater in LDC than in the United States. Early and accurate diagnosis and proper treatment of uterine infections seem to improve the reproductive potential, and thus the profit potential, of cows. Unfortunately, cows often do not have any obvious clinical signs of uterine infections, and many clinicians are unable to accurately diagnose uterine infections. Therefore, this project was conducted to develop and test a new procedure for diagnosing uterine infections. Also, the project was designed to transfer procedures for studying reproductive problems in cattle to scientists in Honduras.

A short review of the literature available at the start of this project will help put the project into proper prospective. In female livestock, reproductive efficiency is the major determinant of lifetime productivity. Ages at puberty and first parturition, and interval from parturition to the next pregnancy (i.e., conception) govern reproductive efficiency. In cattle, nutrition seems to be the primary regulator of ages at puberty and first parturition. Intervals from parturition to ovulation and to estrus, and number of services to conception control the interval from parturition to the next pregnancy.

Endometritis (a type of uterine infection) postpartum reduces reproductive the efficiency of cattle (Fonseca et al., 1983; Coleman et al., 1985; Dobson and Kamonpatana, 1986). Endometritis increases intervals from calving to ovulation, detected estrus, insemination, and conception, and it delays uterine and cervical involution (Fonseca et al., 1983; Coleman et al., 1985). Persistent endometritis causes chronic infertility (Dobson and Kamonpatana, 1986). Uterine infections are not limited to cattle. As many as 30% of the buffaloes examined postpartum (Kahn, 1984; Dobson and Kamonpatana, 1986) and 80% of the buffaloes with delayed uterine involution (Kahn, 1984) had endometritis. Endometritis

accounted for 50% of the reproductive disorders in buffaloes (Samad et al., 1987). Sheep, pigs, and mares also develop uterine infections.

A number of aerobic and anaerobic bacteria have been isolated from uteri of cattle and buffaloes with endometritis (Griffin et al., 1974a; Kahn, 1984; Peter and Bosu, 1987). Uteri harboring pathogenic bacteria other than *Corynebacterium pyogenes* (now called *Actinomyces pyogenes*) had a greater incidence of endometritis than did uteri with nonpathogenic bacteria (Griffin et al., 1974a). However, some uteri harbored known pathogens, such as coagulase-positive staphylococci and beta-hemolytic streptococci, without showing signs of endometritis (Griffin et al., 1974a). When *A. pyogenes* were found in uteri after approximately day 21 postpartum, cows developed severe endometritis and were almost invariably infertile at the first service (Griffin et al., 1974a,b). Based on current literature, *A. pyogenes* seems to be the bacterium consistently associated with severe endometritis.

In cattle, buffaloes, and sheep, concentrations of PGFM in jugular blood plasma begin to increase just before parturition, peak on the day of parturition, and decrease to basal concentrations within approximately 21 days after parturition (Eley et al., 1981; Perera et al., 1981; Lindell et al., 1982; Lewis and Bolt, 1983; Guilbault et al., 1984; Kaker et al., 1984; Lewis et al., 1984; Madej et al., 1984; Fredriksson, 1985; Guilbault et al., 1987; Lewis and Bolt, 1987). The compound, PGFM, is the major stable metabolite of $PGF_{2\alpha}$, and the uterus is a major source of the $PGF_{2\alpha}$ that is detected as PGFM in jugular blood plasma of postpartum cattle and sheep (Guilbault et al., 1984; Lewis and Bolt, 1987). In cattle, changes in PGFM after parturition reflect the rate of uterine and cervical involution (Eley et al., 1981; Lindell et al. 1982; Lewis et al., 1984; Madej et al., 1984; Guilbault et al., 1987). Endometritis delays uterine involution (Fonseca et al., 1983; Coleman et al., 1985) and prolongs the interval from calving to return of PGFM to basal concentrations (Lindell et al., 1982). Ovarian function in postpartum cattle usually does not resume (some period of ovarian inactivity follows pregnancy in livestock) until concentrations of PGFM are basal (Eley et al., 1981; Lindell et al., 1982; Lewis et al., 1984; Majed et al., 1984; Peter and Bosu, 1987). Delays in uterine involution seem to increase the interval from calving to return of normal ovarian function, which is necessary before cows can conceive (Eley et al., 1981; Lindell et al., 1982; Lewis et al., 1984; Majed et al., 1984; Peters, 1984; Peter and Bosu, 1987). Thus, measuring PGFM postpartum may improve detection of uterine problems that reduce reproductive efficiency of cattle.

Even though there seemed to be a relationship between endometritis and concentrations of PGFM in jugular blood plasma, before the present project was conducted, this relationship had been studied in only a small number of cows, and these cows developed endometritis spontaneously (Lindell et al., 1982; Peter and Bosu, 1987). The critical question, does endometritis increase PGFM in jugular blood plasma, had not been answered. The most meaningful way to answer that question experimentally is to induce endometritis in postpartum animals and then measure changes in PGFM in response to the infection. Based on the literature reviewed, induction of endometritis should increase PGFM concentrations in jugular plasma, and PGFM should be a useful method for improving diagnosis of endometritis in bovine animals in LDC.

Before measurements of PGFM can be used to practically diagnose uterine infections in lesser developed countries, one must develop a rapid, simple, reliable, and environmentally-safe method for measuring PGFM; enzymeimmunoassay should meet these needs better than radioimmunoassay (RIA). Indeed, the use of RIA for measuring various hormones in LDC has been limited because of the lack of adequate laboratory facilities and personnel trained to ensure the safe use of radioisotopes, lack of adequate procedures for disposing of radioactive wastes, high cost of reagents, and high cost of buying and maintaining sophisticated equipment for measuring radioactivity (Davina et al., 1986). In all countries, RIA is not suitable for field use. Use of EIA avoids most of the problems associated with the use of RIA.

In summary, reproductive efficiency is the major determinant of lifetime productivity of cattle. Endometritis reduces reproductive efficiency of cattle. Endometritis delays uterine involution, which usually precedes the postpartum return of normal ovarian function. Changes in jugular venous plasma concentrations of PGFM in postpartum cattle reflect the rate of uterine involution. Endometritis seems to delay the postpartum return of PGFM to basal concentrations, but the effects of endometritis on concentrations of PGFM had not been tested experimentally. Measuring changes in PGFM postpartum may improve the diagnosis of endometritis and is a method for monitoring uterine involution postpartum. To be a practical scientific and clinical tool in lesser developed countries, PGFM should be measured with EIA. The EIA can be used in programs to improve the production potential of cattle in lesser developed countries. Countries, such as Honduras, could especially benefit, because their cattle industries are advanced enough to take advantage of modern technologies.

Therefore, we set out to 1) to determine whether induced and spontaneous endometritis increase the concentrations of PGFM in jugular blood plasma from postpartum cattle, 2) develop an EIA to measure PGFM in blood plasma from cows, and 3) use the EIA in Honduras to determine whether changes in concentrations of PGFM can be used to improve diagnosis of endometritis and monitor the rate of uterine involution in cattle.

Scientifically, this project allowed us to establish experimentally that uterine infections (i.e., endometritis) increase PGFM concentrations in jugular venous blood. It also allowed us to establish that progesterone from the first corpus luteum after calving, or a progestogen (i.e., synthetic progesterone-like compound), increases the susceptibility of the uterus to infections and permits the uterus to secrete increased amounts of $PGF_{2\alpha}$, which are probably detected as PGFM in jugular blood. This added important information to the literature on uterine infections and gave us the background information that we needed to conduct studies with sheep to determine how the uterine immune system prevents or controls uterine infections. An Egyptian student working with me (G. S. Lewis) just finished his Ph.D. degree and returned to Egypt, and a new student from Egypt is now working with me on his Ph.D. The subject of both students' research is uterine immune response to infections; the funding for both students' research is from other contracts.

Practically, this project allowed us to develop and deploy a new diagnostic tool. The project also allowed us to transfer to Honduras a considerable amount of information on



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detecting and managing uterine infections in cattle. This information is being used to improve herd health and to teach students currently accepted clinical practices.

## MATERIALS AND RESULTS:

Our first objective was to develop and validate an EIA for PGFM. To develop the EIA, we were able to convince Dr. K. M. Maxey (Cayman Chemical, Ann Arbor, MI) to produce the tracer that we needed; Cayman Chemical holds the use patent for the reagents needed to develop the EIA tracer with the characteristics that were required for this project. The tracer is acetylcholine esterase linked to 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM). Using the tracer and a number of other reagents, we developed a valid and extremely sensitive EIA to measure PGFM (Del Vecchio et al., 1992b), the stable metabolite of $PGF_{2\alpha}$; $PGF_{2\alpha}$ is produced in the uterus in response to various stimuli. The EIA is simple, environmentally safe, and suitable for use in lesser developed countries, because no radioactive or other hazardous materials are used in the assay. Our data indicate clearly that our EIA for PGFM is superior, in terms of speed, reliability, sensitivity, and safety, to our radioimmunoassay for PGFM. For all of the details of the assay, see Del Vecchio et al. (1992d; in Appendix). NOTE: Dr. R. P. Del Vecchio was a postdoctoral fellow that I (G. S. Lewis) assigned to the project. He was responsible for the day-to-day work on the portion of the project conducted at Virginia Tech.

Our second objective was to determine experimentally whether induced uterine infections increased jugular venous concentrations of PGFM. Based on observational evidence, but no experimental evidence, uterine infections alter the postpartum changes in PGFM concentrations in cattle; this probably reflects the effect of pathogenic organisms on uterine secretion of $PGF_{2\alpha}$. So for this study, we isolated *Actinomyces pyogenes* and *Escherichia coli* from a cow with a severe uterine infection. The organisms were produced in large amounts and used to induce uterine infections in cows. Our data indicate that we were able to induce uterine infections in the experimental cows. The uterine infections increased PGFM concentrations in jugular blood. Those results supported our original hypothesis and convinced us that we should be able to use jugular PGFM concentrations, measured with our EIA, to improve the diagnosis of uterine infections. For all of the details of the experiment, see Del Vecchio et al. (1992c; in Appendix).

While attempting to induce uterine infections, we discovered that cows must be exposed to progesterone before we were able to detect an increase in jugular PGFM concentrations. Indeed, the literature indicates that the most common types of uterine infections (endometritis and pyometra) are not likely to occur in postpartum cows until after they have ovulated and the corpus luteum, which forms at the site of ovulation, begins to produce progesterone. Therefore, we conducted a study with prepubertal heifers to determine whether their uteri would respond to exogenous stimulation before that they had been exposed to progesterone. The results of that study indicate that the uterus must be "primed" with a progestogen before we should expect to detect an infection-induced increase in jugular PGFM concentrations. For all of the details of the experiment, see Del Vecchio et al. (1992a; in Appendix).

Our final objective for the work at Virginia Tech was to determine whether spontaneous uterine infections altered jugular PGFM concentrations. The results of this study indicate that uterine infections that develop spontaneously after cows form their first corpus luteum after calving increase jugular venous concentrations of PGFM. For all of the details of the experiment, see Del Vecchio et al. (1992b; in Appendix).

Overall, the data from the Virginia Tech portion of this project indicate that regardless of whether uterine infections were induced or spontaneous after cows developed their first postpartum corpus luteum, the infection increased jugular PGFM concentrations. Based on the Virginia Tech portion of the project we believe that jugular PGFM, measured with our environmentally-safe EIA, can be used to improve the diagnosis of uterine infections in postpartum cows with corpora lutea.

Our objectives for the LDC portion of the project were to determine whether 1) spontaneous uterine infections (i.e., endometritis) increase jugular concentrations of PGFM in postpartum cattle in Honduras, 2) treatment for endometritis affects PGFM concentrations, and 3) concentrations of PGFM can be used alone or in combination with other variables to improve diagnosis of endometritis in postpartum cows. The data (see data in Appendix for individual cows) for this portion of the study have been collected and analyzed several times.

The overall analysis of variance indicated that treatment (i.e., clinically normal cows, cows with uterine infections that were treated with Lutalyse [$PGF_{2\alpha}$] as soon as the infections were diagnosed, cows with uterine infections that were not treated until 14 days after the infections were diagnosed) did not affect the average concentration of PGFM, and there was no treatment X time interaction. However, after a considerable amount of work with the data and using several different statistical approaches, I (G. S. Lewis) am not satisfied with the outcome of the analyses. There are several reasons for that. 1) Typical time-trend analyses, which I used initially, prevent us from detecting short-term changes in PGFM. Based on Del Vecchio et al. (1992c), the short-term changes in PGFM seem to be the most informative. Recently, I obtained a software package to better evaluate the short-term changes in PGFM for each cow, but I have not yet worked out the appropriate programming. 2) Based on the times postpartum and bacteriological data, many of the cows seem to have developed puerperal metritis, rather than endometritis; the clinician that examined the cows for Dr. Matamoros did not discriminate between the two types of uterine infections. I am in the process of evaluating each record for each cow and classifying each cow within each uterine infection group to puerperal metritis and endometritis subgroups. Next, I will use the new software to evaluate specific characteristics of the changes in PGFM for each cow. After all of the reclassifications, etc. are complete, analyses of variance will be used to reanalyze the various types of data that will be generated. My concern is that creating subgroups will partition the degrees of freedom into groups that are too small to test our original hypotheses. When I am satisfied that the data have been analyzed appropriately (i.e., bias due to type of infection has been accounted for), we will prepare a manuscript and submit it to a refereed journal for the reviewers and editor to consider for publication.

In spite of the problems with reclassifying the data from Honduras, the PGFM EIA used for this project is environmentally safe and reliable enough to be used in lesser developed countries. Indeed, we believe that, based on our work in Honduras, the EIA is ideally suited for use in developing countries to improve, based mainly on our work at Virginia Tech, the diagnosis of one type of uterine infection and reproductive management of cows. The problem of clinicians failing to discriminate between various types of uterine infections taught us that we must consider the time after calving as we further evaluate the field use of PGFM to diagnose uterine infections.

## IMPACT, RELEVANCE, AND TECHNOLOGY TRANSFER:

The data, and experience, from this project will improve reproductive management of cows at the Escuela Agricola Panamericana. Dr. Matamoros will also use the data and experience to improve reproductive management in commercial herds of cattle in the region around the Escuela Agricola Panamericana; he conducts outreach programs. In addition, and perhaps most importantly, the data and experience will be used to teach students at the Escuela Agricola Panamericana how to use technological advances to improve livestock management. Those students will take that knowledge to several countries in Latin America. Thus, the knowledge from this project is likely to be amplified several fold throughout Latin America.

In addition, this project allowed us to estabiish in Honduras a fully functional reproductive endocrinology laboratory for farm animals. This is the first such laboratory in Honduras, and one of the first in Central America. This laboratory will enhance the research capabilities of Dr. Matamoros, his department, and the Escuela Agricola Panamericana. In addition, this project allowed us, at Virginia Tech, to develop the background information needed to develop meaningful Ph.D. programs for students from Egypt. Those programs are designed to understand how the uterine immune system prevents or controls uterine infections, which are a major problem in Egypt. Thus, the capabilities that this project gave us will be amplified in Egypt.

The immediate use of the results from this project will be to develop additional research programs to increase our understanding of uterine infections. Also, a number of scientists are using our EIA protocol to phase out their radioimmunoassays; they are looking for ways to reduce their use of radioactive materials. However, the clinical use of the PGFM EIA to diagnose uterine infections is progressing slowly. Clinicians must become convinced that this procedure will substantially enhance their ability to treat cows, and that is an inherently slow process. To speed the process of convincing clinicians, additional and larger field studies are needed to assess the use of PGFM data compared with traditional methods for diagnosis uterine infections. Those studies must include training to teach clinicians how to diagnose the specific types of uterine infections and use the EIA appropriately.

In summary, this project substantially improved the research capabilities of Dr. Matamoros in Honduras and those of my (G. S. Lewis) laboratory at Virginia Tech. The experience and knowledge gained from this project, and subsequent projects, will be amplified throughout Latin America, Egypt, and the United States.

**PROJECT ACTIVITIES/OUTPUTS:**

**Training:**

Dr. Matamoros visited my laboratory from February 15 to 22, 1993. While Dr. Matamoros was here, we taught him several of the procedures that he now uses to collect various types of samples from cows. Also, we taught him several laboratory procedures that have been transferred to his laboratory.

I (G. S. Lewis) visited Dr. Matamoros from April 24 to May 8, 1994. I set up the PGFM and a progesterone EIA, trained people to conduct the assays, and participated in measuring PGFM and progesterone in the blood samples collected for this project. When I left Honduras, Dr. Matamoros had a fully functional reproductive endocrinology laboratory.

Meetings attended to present results from this project:

Annual Meeting Northeast Regional American Society of Animal Science-American Dairy Science Association, 1991.

Annual Meeting Southern Section of the American Society of Animal Science, 1992.

Annual Meeting of the American Society of Animal Science, 1992.

**Publications:**

Del Vecchio, R. P., E. E. Custer, W. E. Beal, and G. S. Lewis. 1992. Jugular plasma concentrations of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ in prepubertal beef heifers treated with progestogen then challenged with oxytocin. Prostaglandins 44:509-518.

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**PROJECT PRODUCTIVITY:**

All but one of the objectives of the project was accomplished. We had planned to validate the PGFM EIA for milk samples. Because of the chemical and physical properties of milk, it created a number of difficulties. Blood samples are so easy to collect and process through the assay that we decided that the cost of solving the problems with milk would exceed the any benefits from using milk samples.

**FUTURE WORK:**

As described before, this project has already lead to additional and more in-depth research on uterine infections at Virginia Tech; that work is scheduled to continue for several years. Dr. Matamoros and I would like to continue our collaboration, but we have not yet located a source of support. Without additional support, the future of the endocrine laboratory in Honduras is not secure; endocrine work is expensive, and Dr. Matamoros will have trouble supporting the laboratory with his small budget from the Escuela Agricola Panamericana.

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**APPENDIX:**

**KEY TO FIGURES FOR INDIVIDUAL COWS IN HONDURAS**

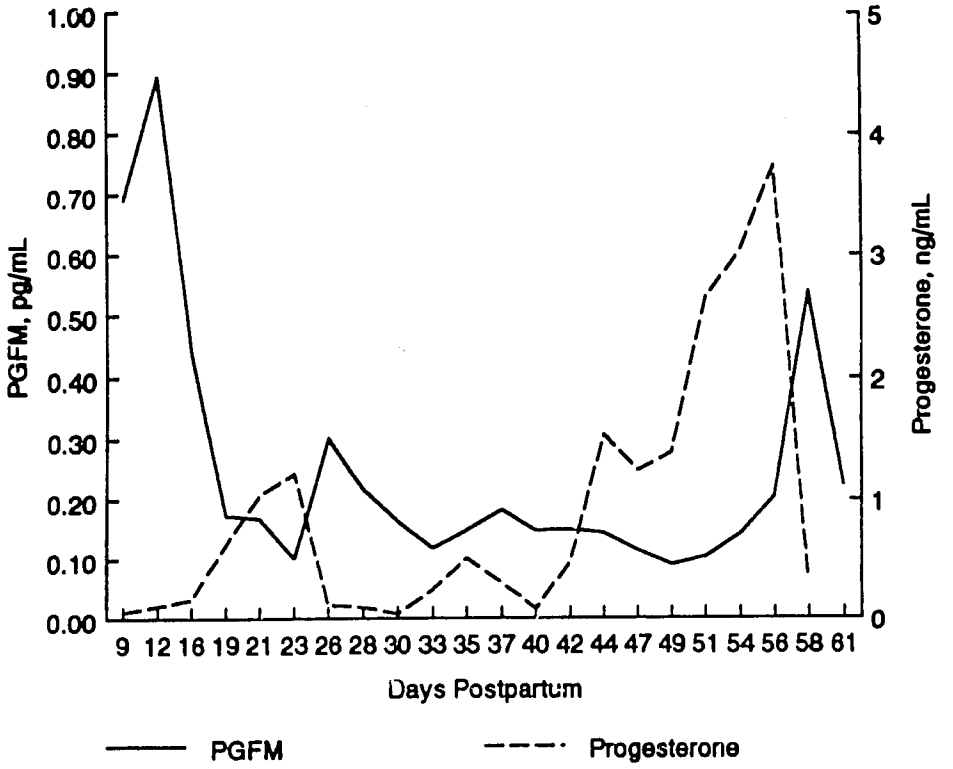
| Treatment[a] | | |
|---|---|---|
| 1 | 2 | 3 |
| - - - - - - - - - - - - Cow number - - - - - - - - - - - - | | |
| 13189 | 31791 | 14689 |
| 18885 | 32989 | 16884 |
| 19190 | 34889 | 21588 |
| 34690 | 46285 | 30388 |
| 35986 | 46786 | 30491 |
| 36188 | 311590 | 35990 |
| 39890 | 312990 | 41291 |
| 47191 | 313390 | 42084 |
| 314889 | 413489 | 114489 |
| 415089 | 712688 | 711889 |
| 713387 | | |

[a]Treatment 1 = clinically normal cows.

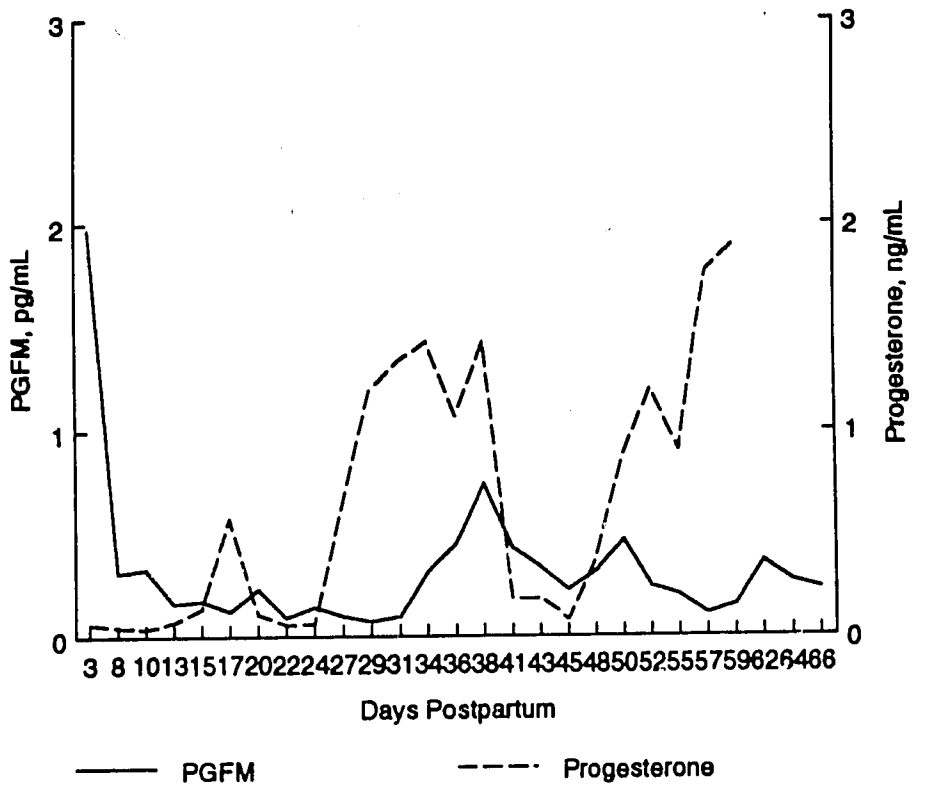
Treatment 2 = cows with uterine infections were treated with Lutalyse ($PGF_{2\alpha}$) as soon as the infections were diagnosed.

Treatment 3= cows with uterine infections were not treated until 14 days after the infections were diagnosed.

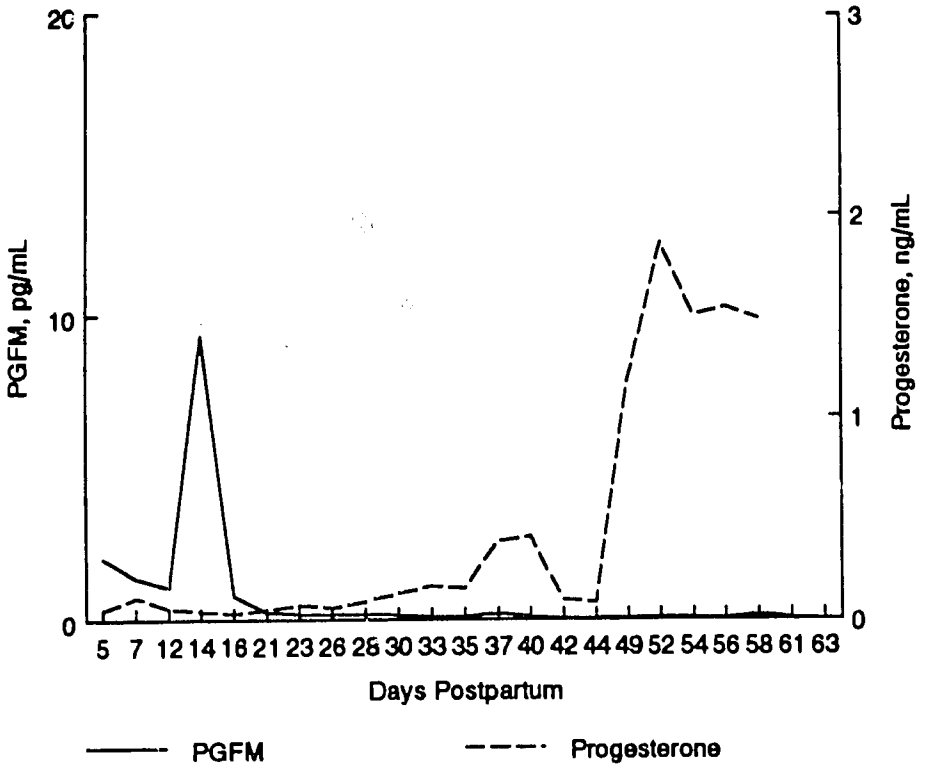
Cow #13189



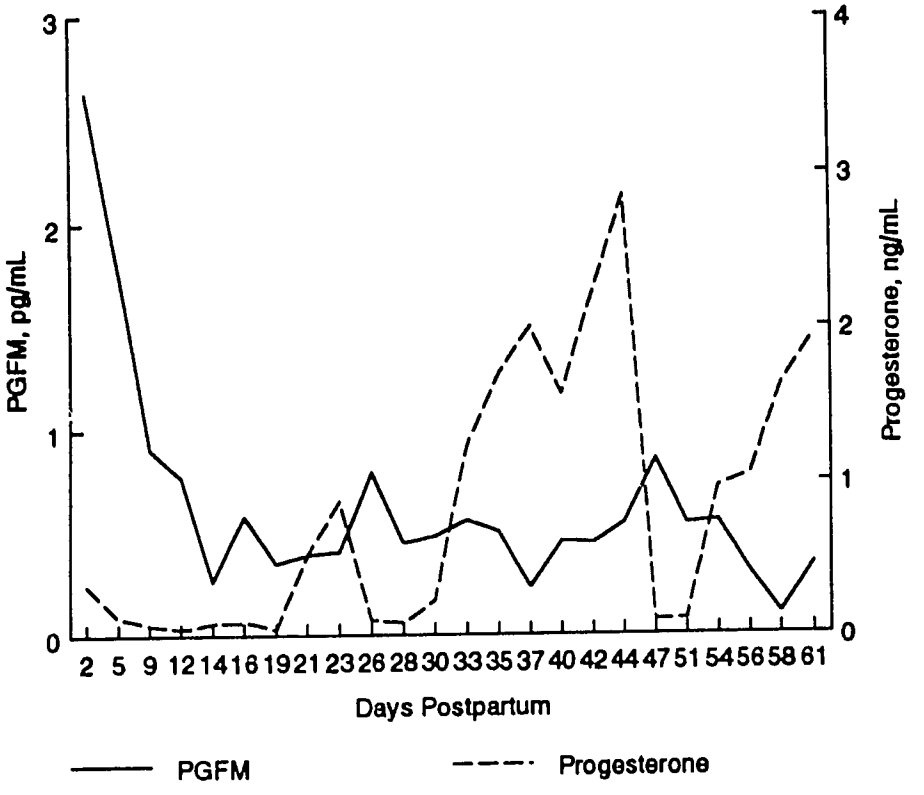

Cow #14689

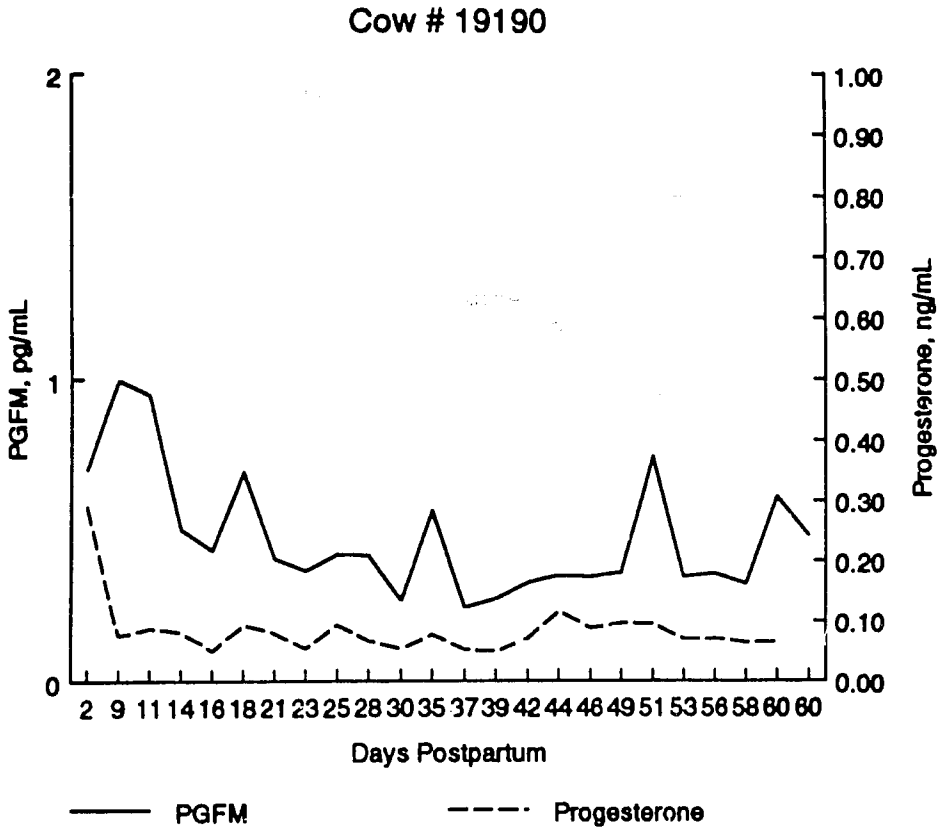


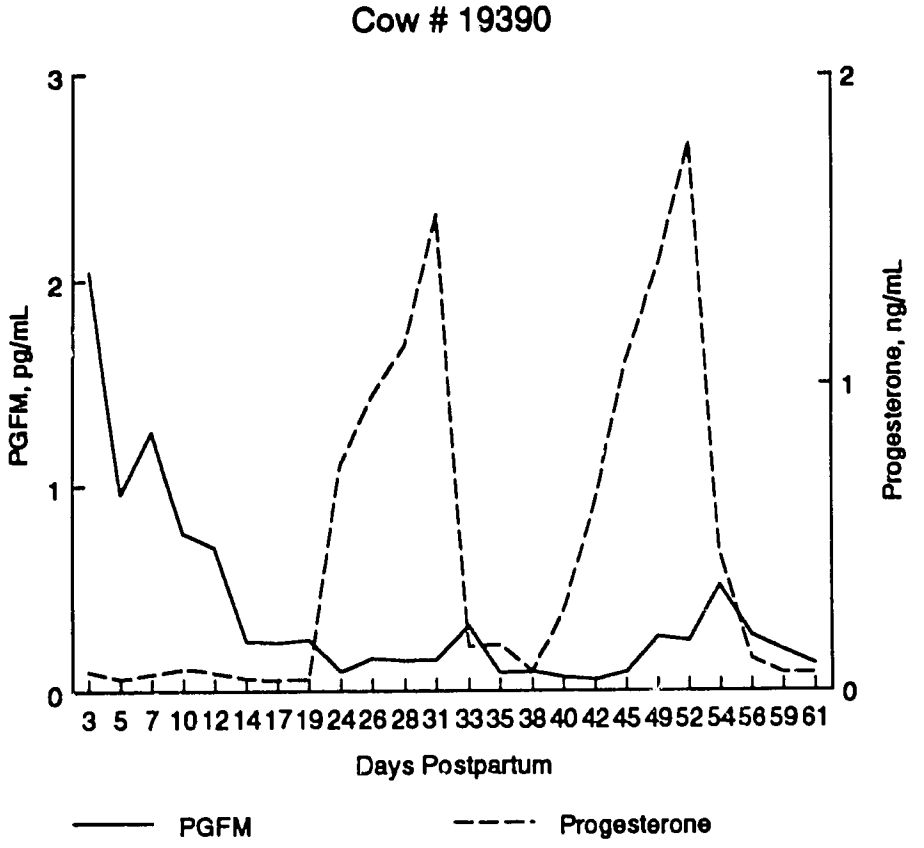
Cow # 16884

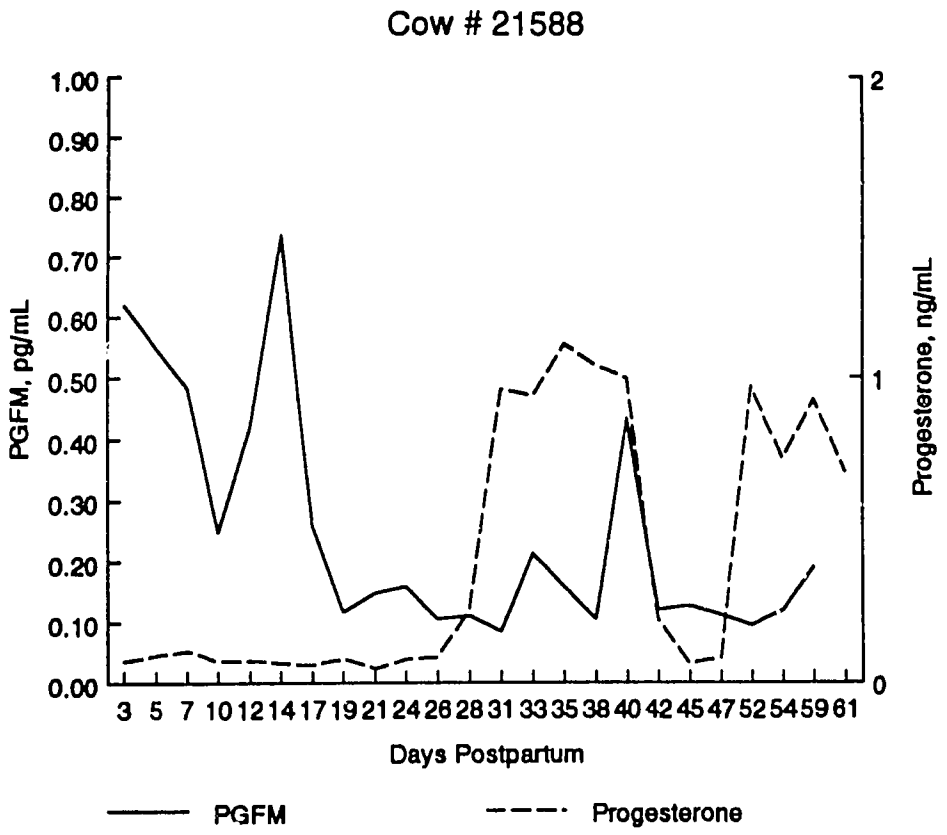


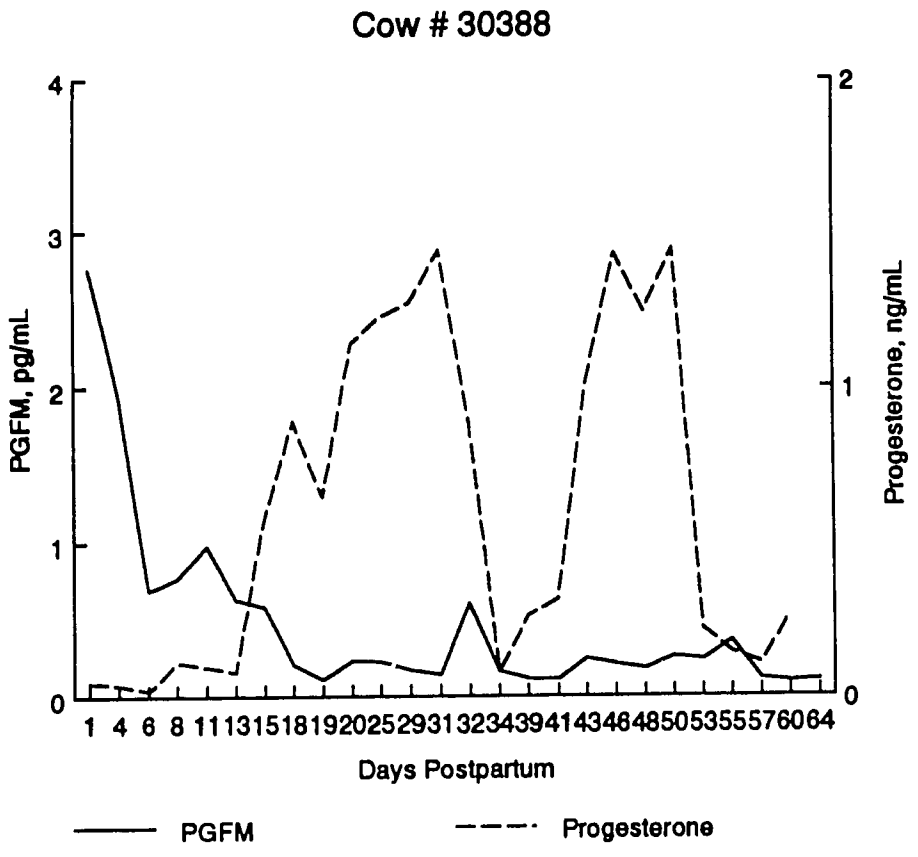

Cow # 18885



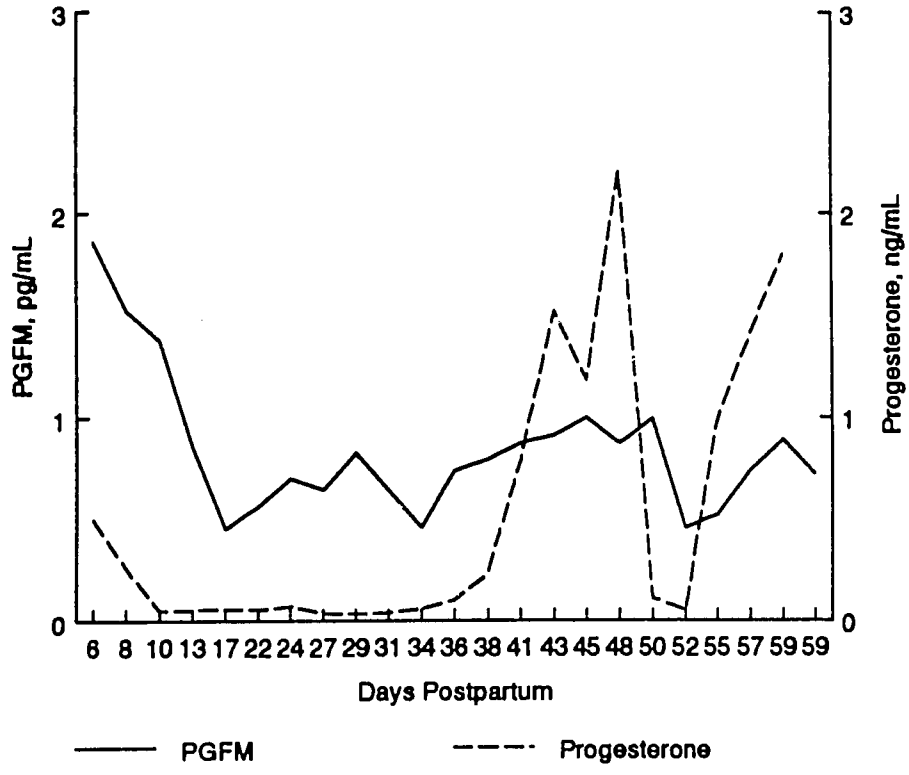

Cow # 19190
PGFM, pg/mL
Progesterone, ng/mL
2
1
0
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
2 9 11 14 16 18 21 23 25 28 30 35 37 39 42 44 46 49 51 53 56 58 60 60
Days Postpartum
PGFM
Progesterone


Cow # 19390
PGFM, pg/mL
Progesterone, ng/mL
3
2
1
0
2
1
0
3 5 7 10 12 14 17 19 24 26 28 31 33 35 38 40 42 45 49 52 54 56 59 61
Days Postpartum
PGFM
Progesterone

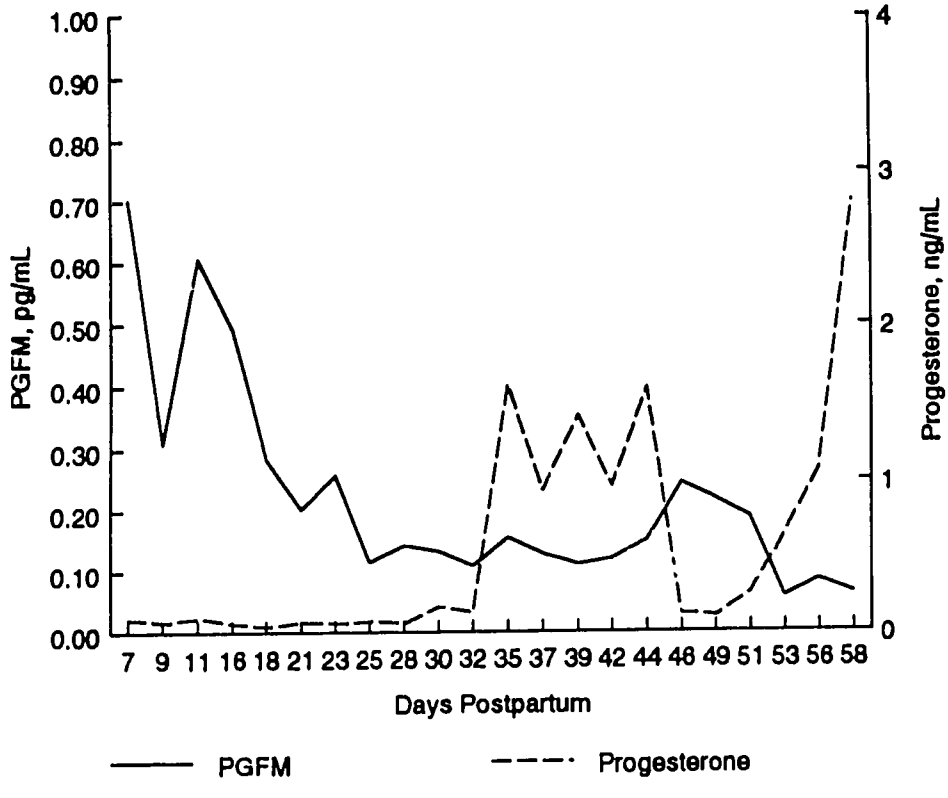

Cow # 21588
PGFM, pg/mL
Progesterone, ng/mL
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
2
1
0
3 5 7 10 12 14 17 19 21 24 26 28 31 33 35 38 40 42 45 47 52 54 59 61
Days Postpartum
PGFM
Progesterone


Cow # 30388
PGFM, pg/mL
Progesterone, ng/mL
4
3
2
1
0
2
1
0
1 4 6 8 11 13 15 18 19 20 25 29 31 32 34 39 41 43 46 48 50 53 55 57 60 64
Days Postpartum
PGFM
Progesterone

Cow # 30491




Cow # 31791



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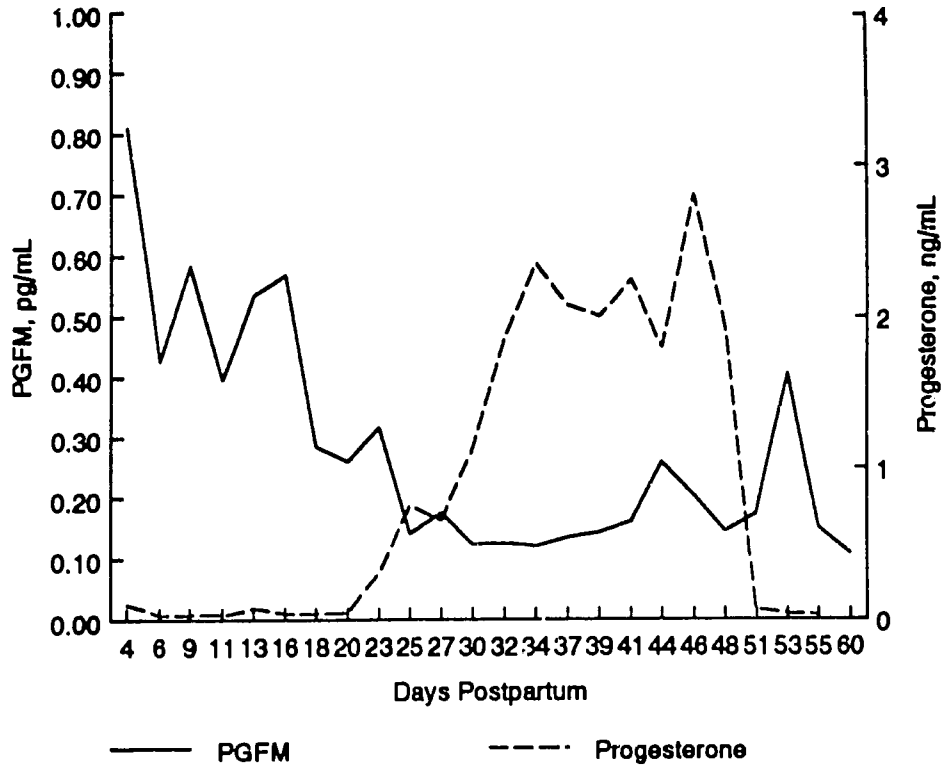
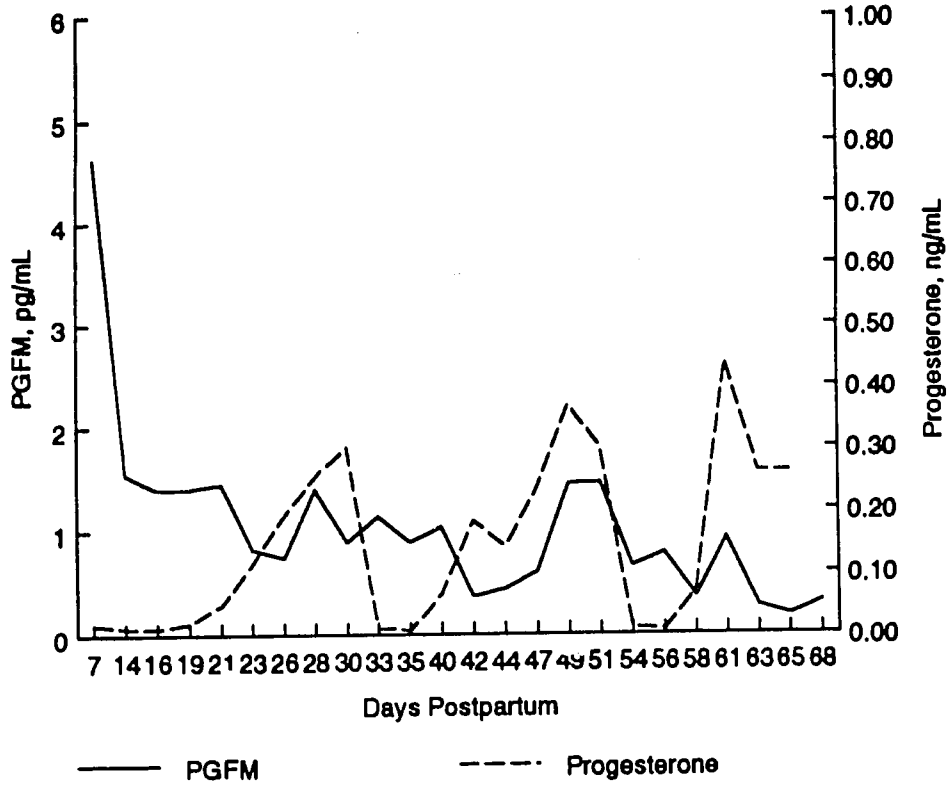
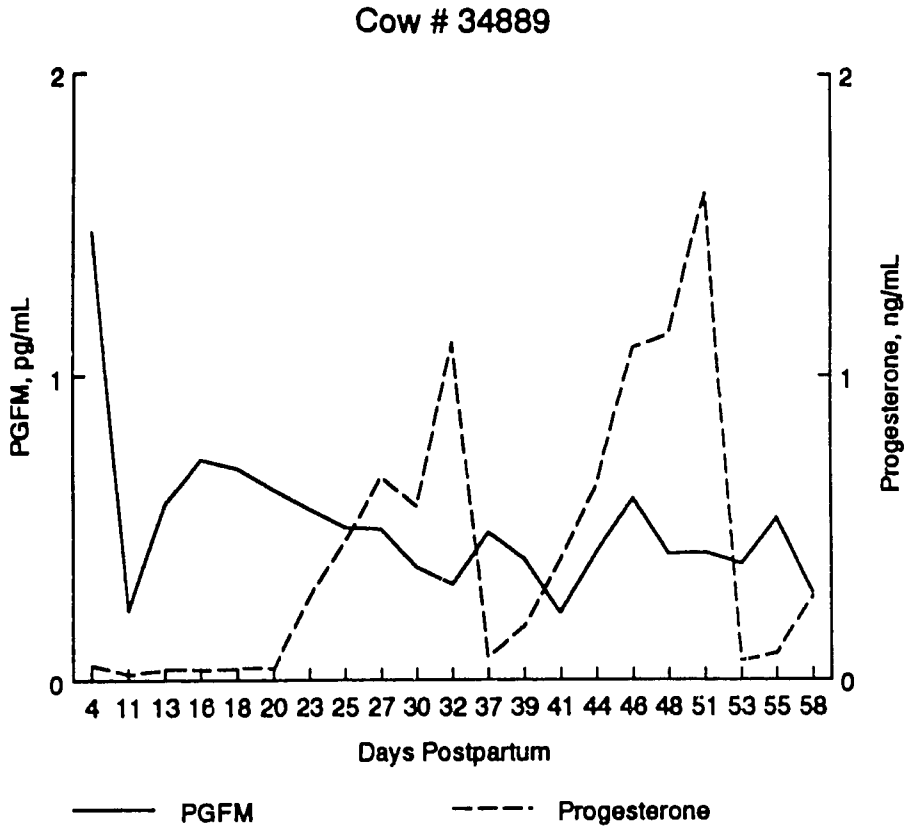
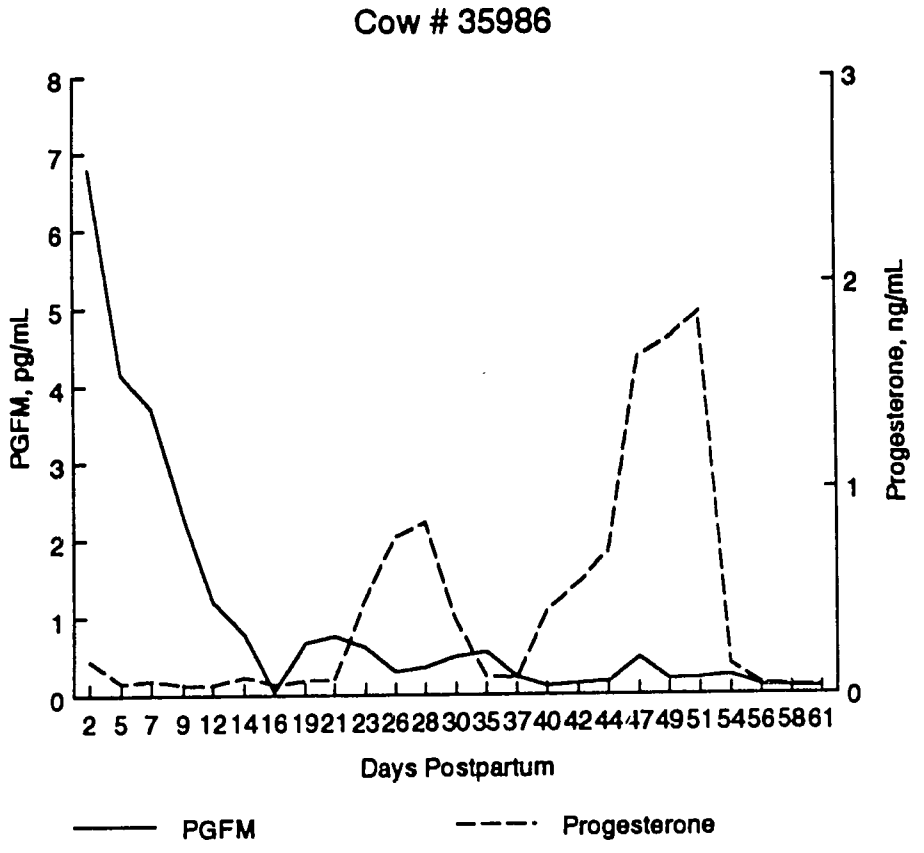



Cow # 34690

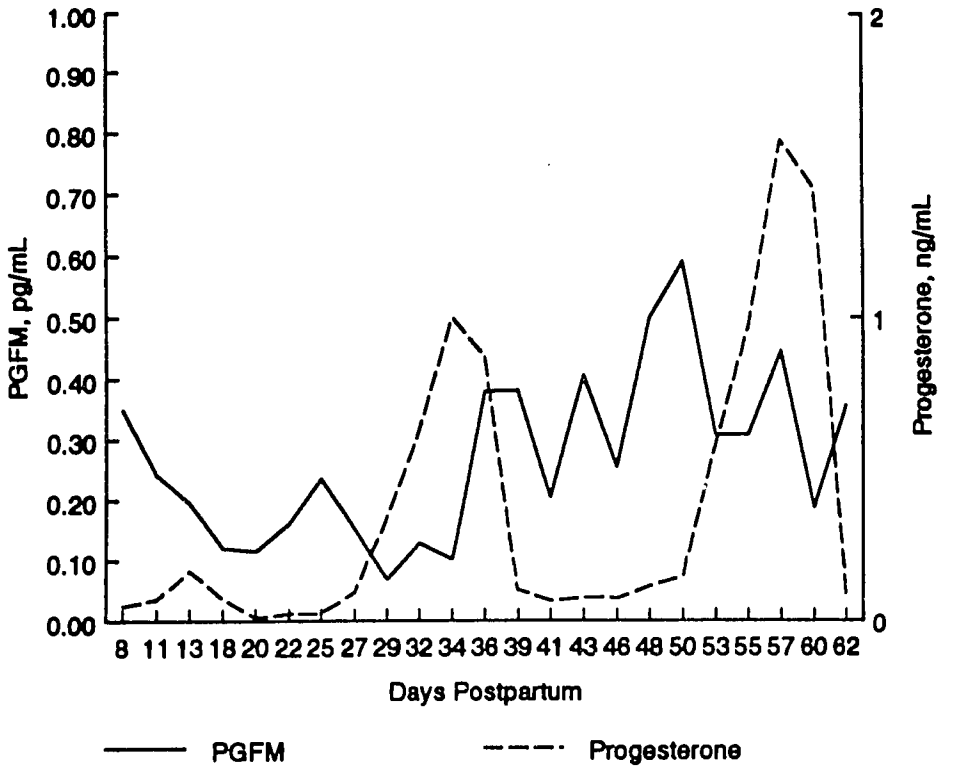



Cow # 34889
PGFM, pg/mL
Progesterone, ng/mL
2
1
0
4 11 13 16 18 20 23 25 27 30 32 37 39 41 44 46 48 51 53 55 58
Days Postpartum
PGFM
Progesterone


Cow # 35986
PGFM, pg/mL
Progesterone, ng/mL
8
7
6
5
4
3
2
1
0
3
2
1
0
2 5 7 9 12 14 16 19 21 23 26 28 30 35 37 40 42 44 47 49 51 54 56 58 61
Days Postpartum
PGFM
Progesterone

20

Cow # 35990

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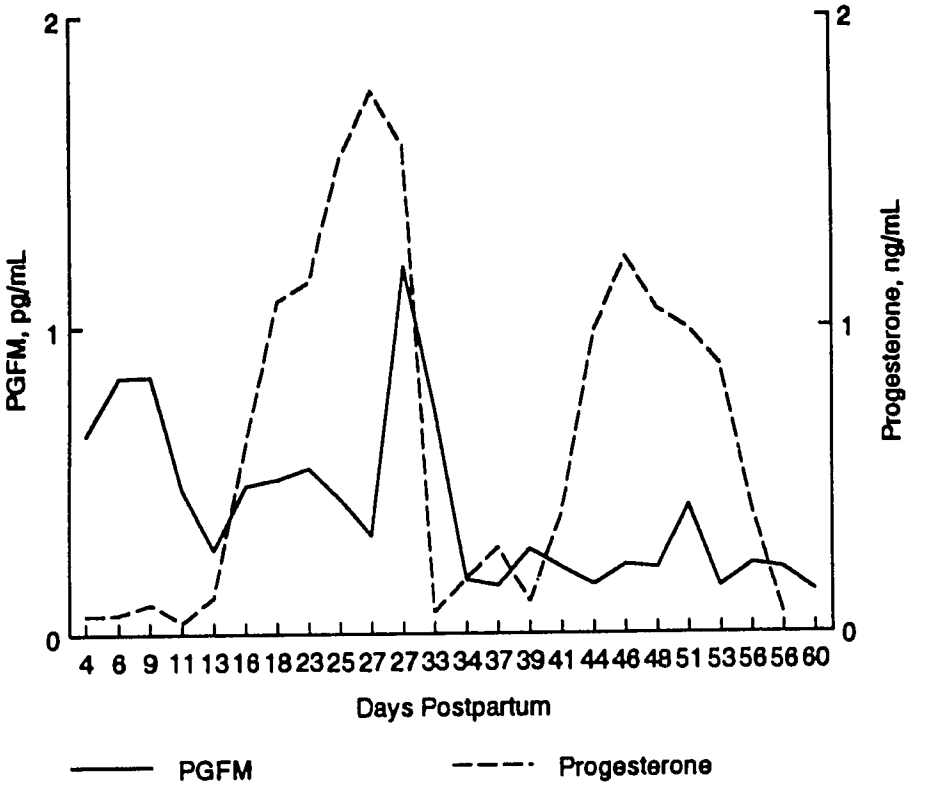
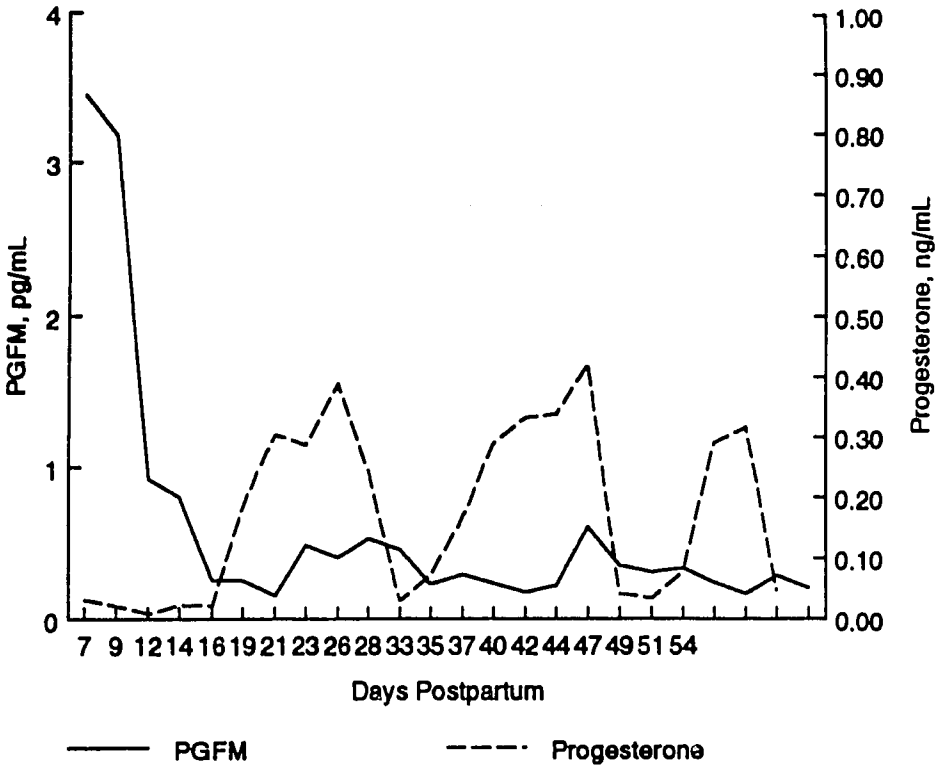


Cow # 39890

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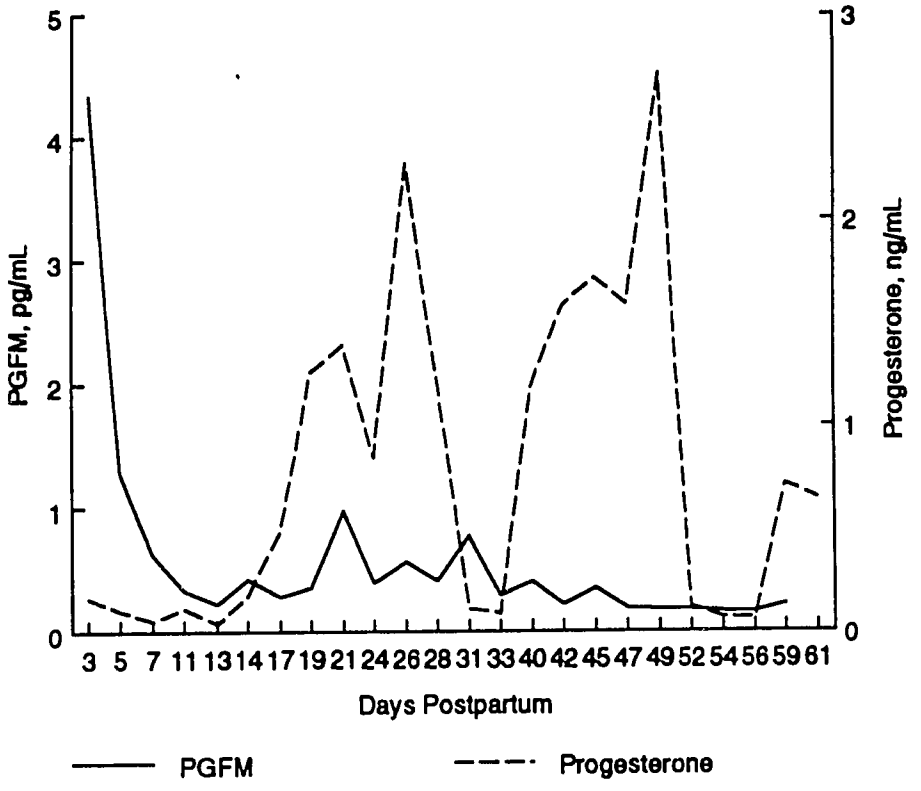

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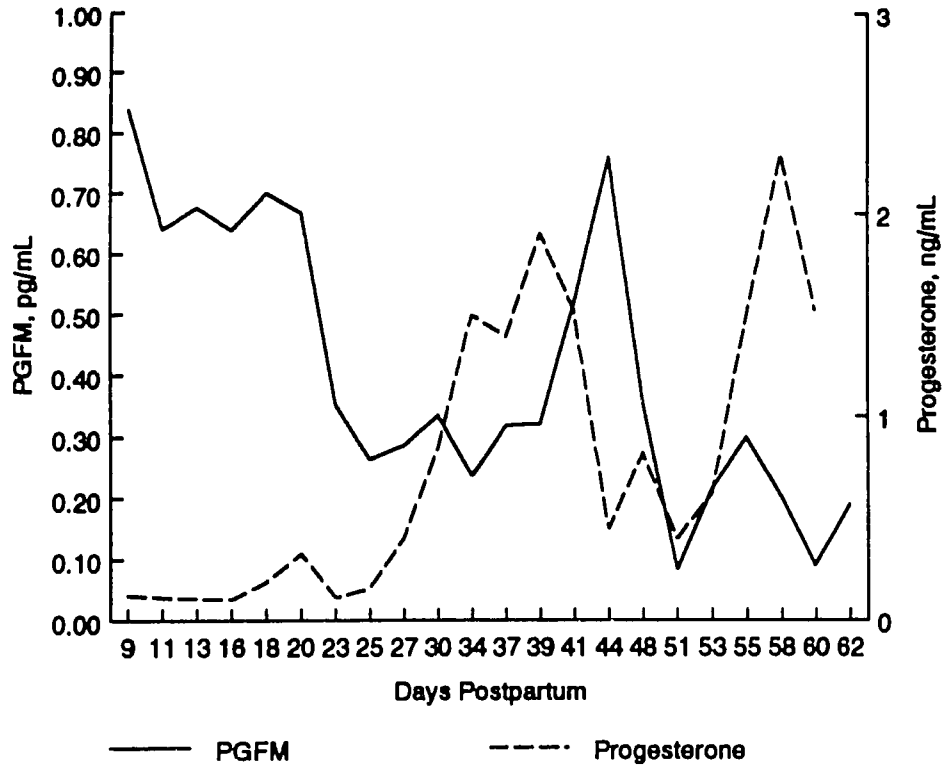


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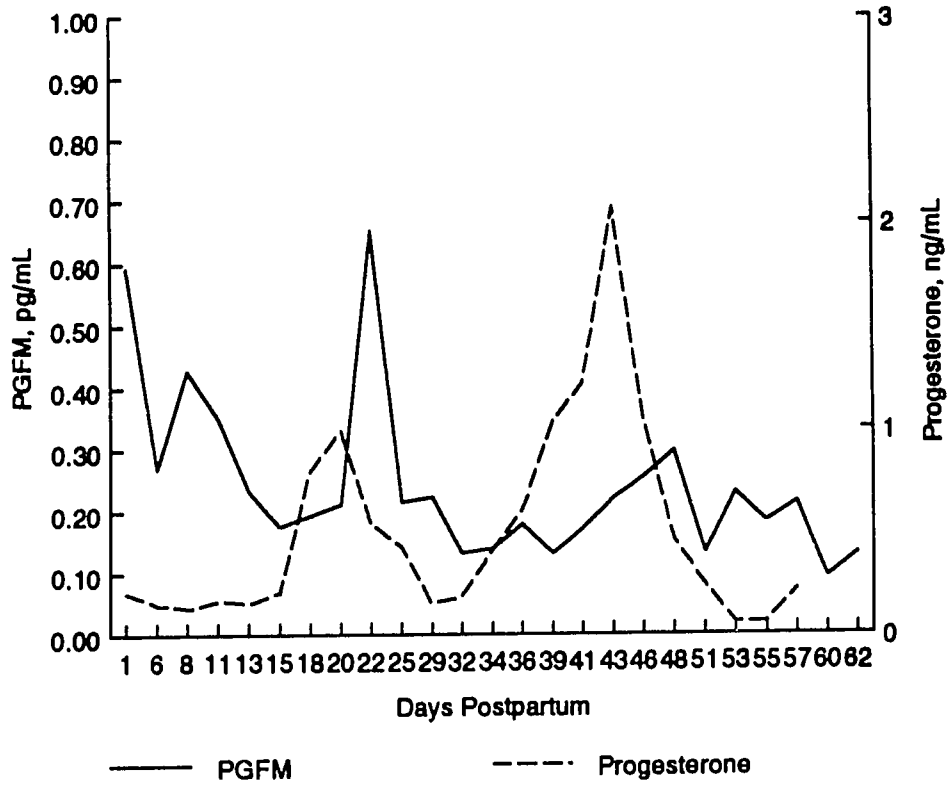

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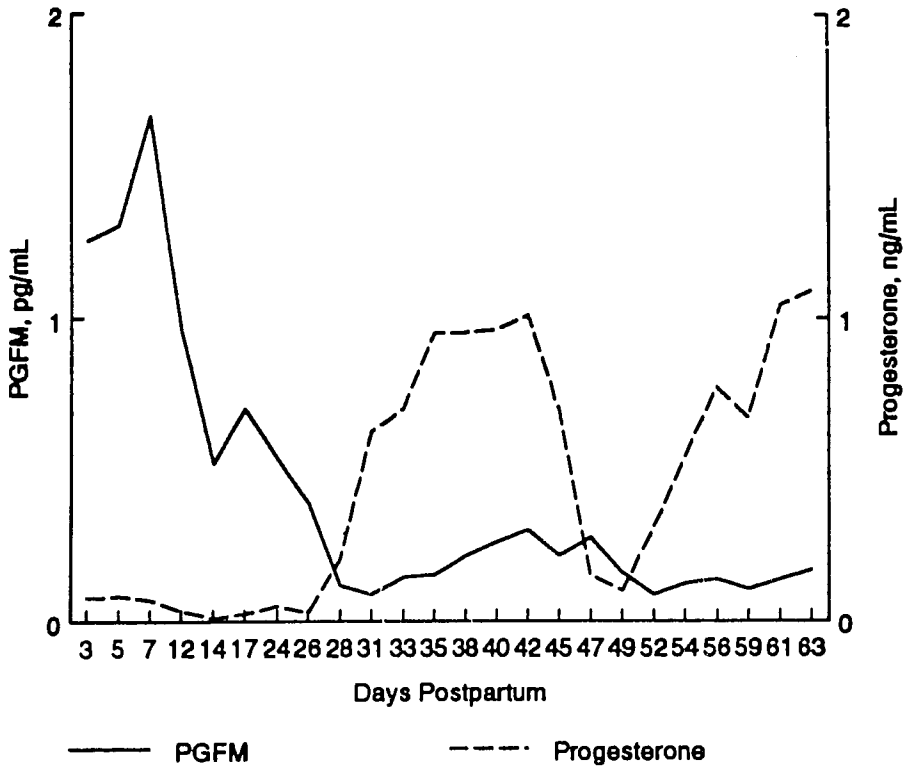


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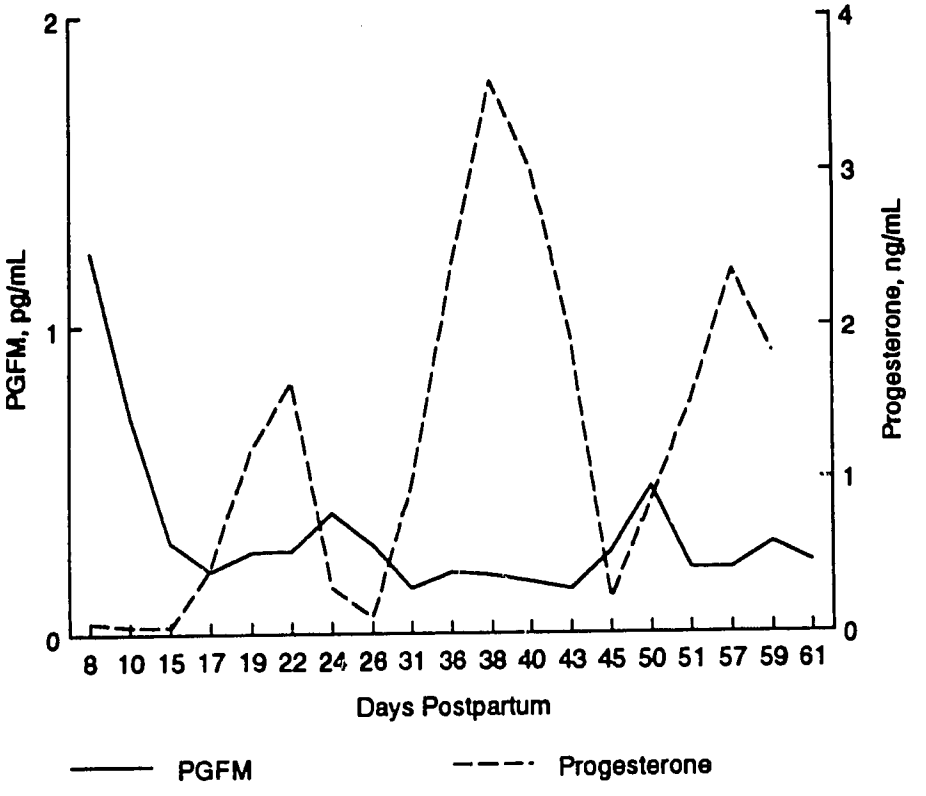
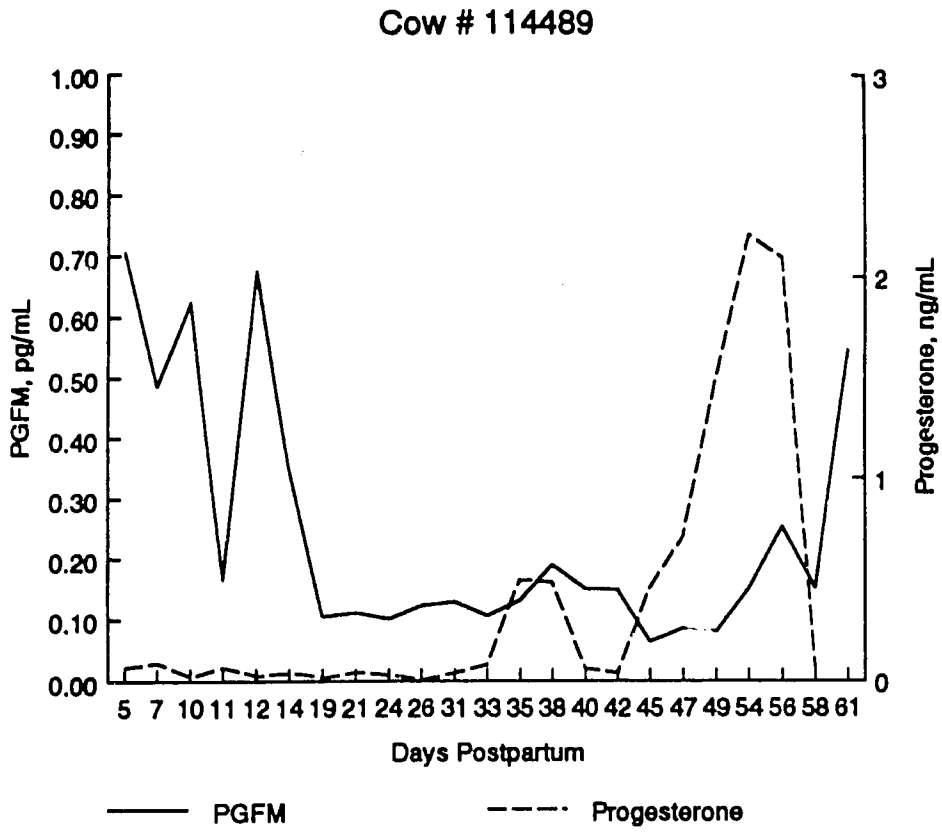
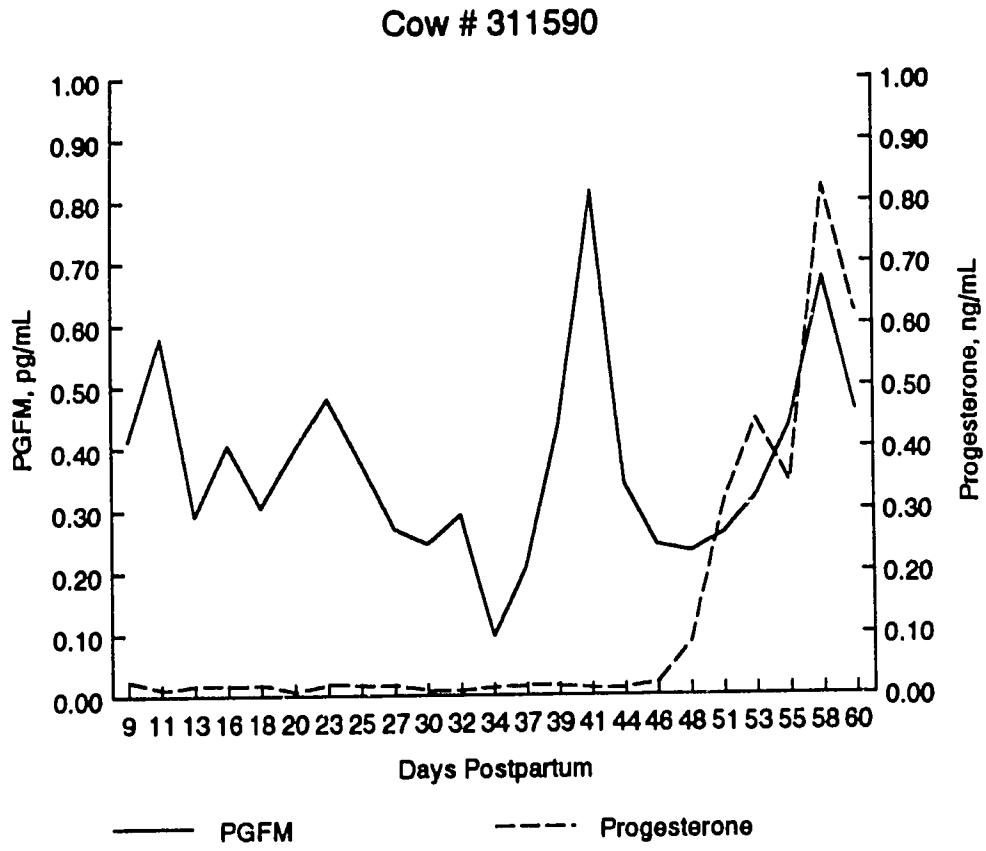
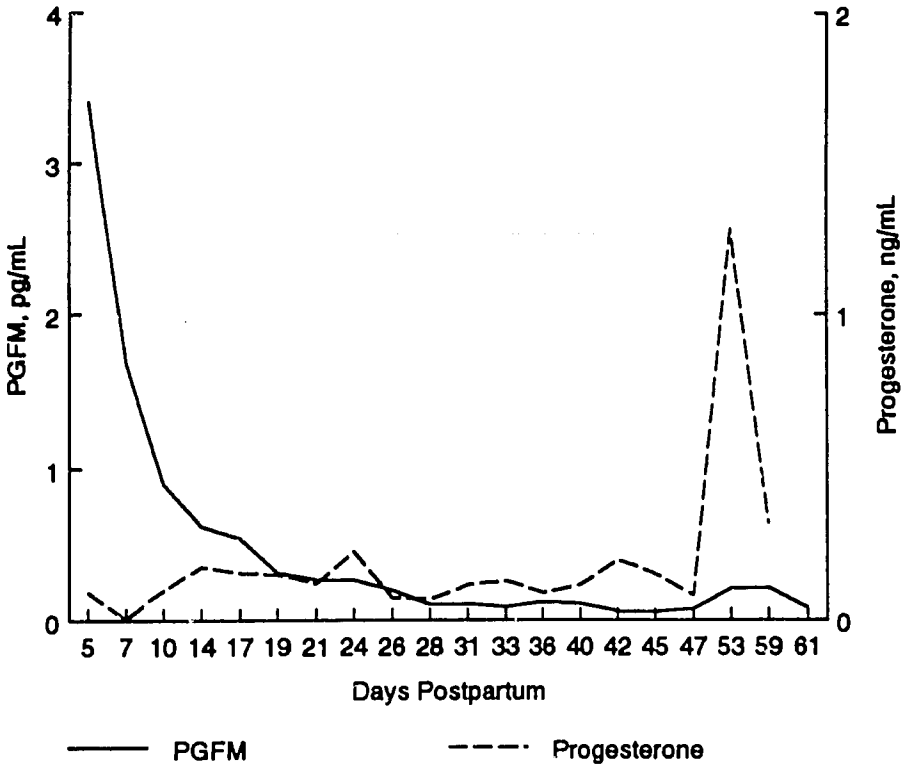



Cow # 114489
PGFM, pg/mL
Progesterone, ng/mL
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
3
2
1
0
5 7 10 11 12 14 19 21 24 26 31 33 35 38 40 42 45 47 49 54 56 58 61
Days Postpartum
PGFM
Progesterone


Cow # 311590
PGFM, pg/mL
Progesterone, ng/mL
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
9 11 13 16 18 20 23 25 27 30 32 34 37 39 41 44 46 48 51 53 55 58 60
Days Postpartum
PGFM
Progesterone

Cow # 312990

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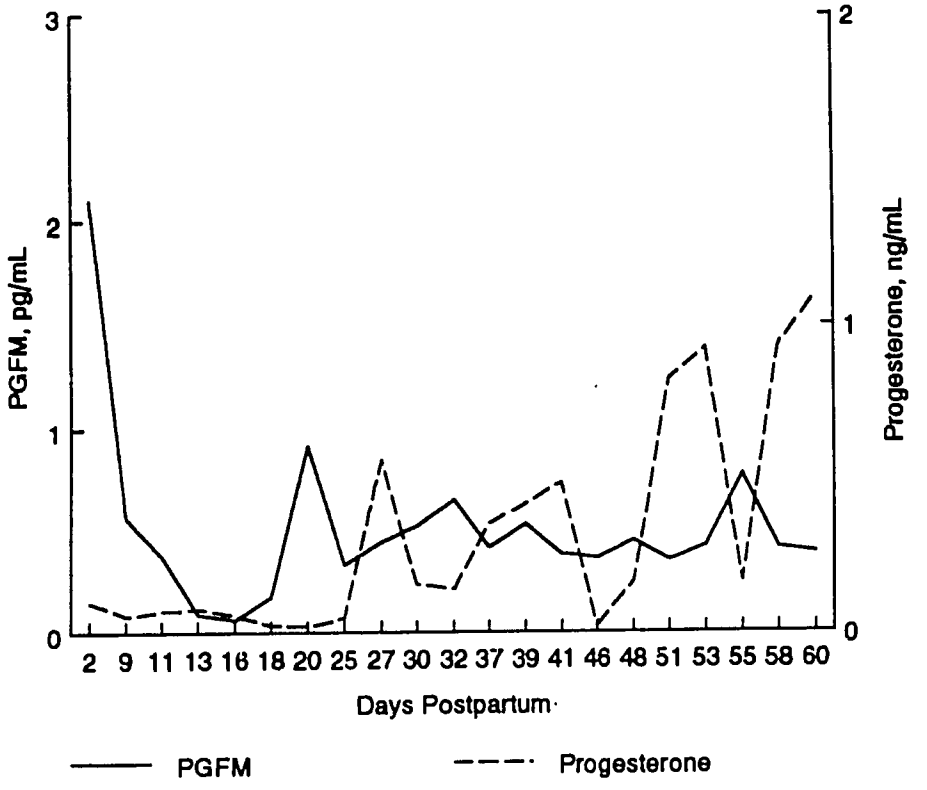

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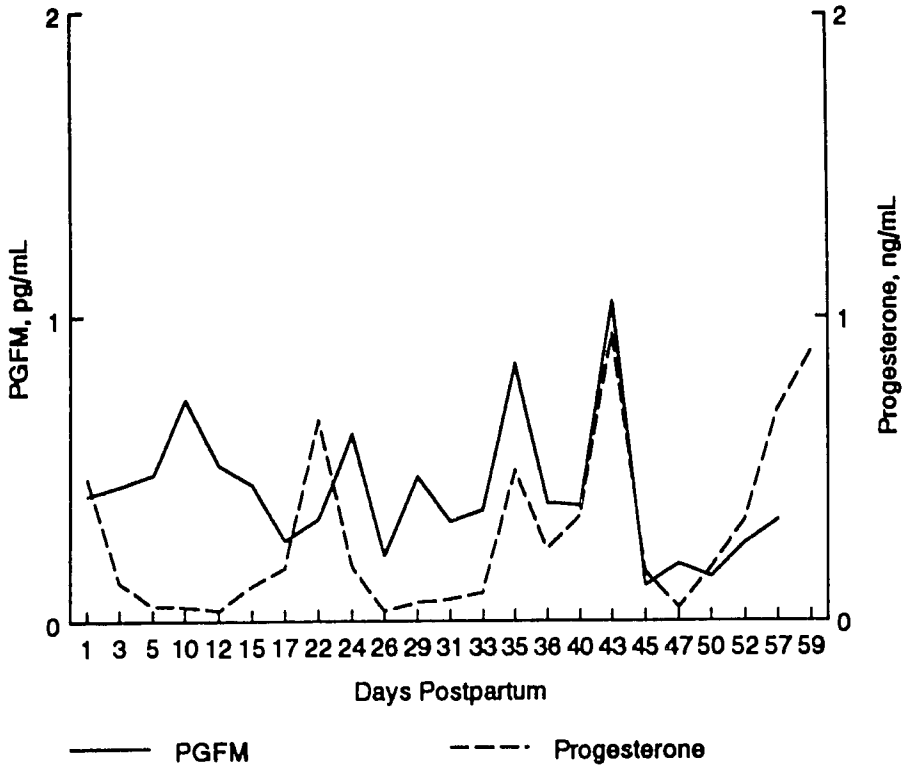


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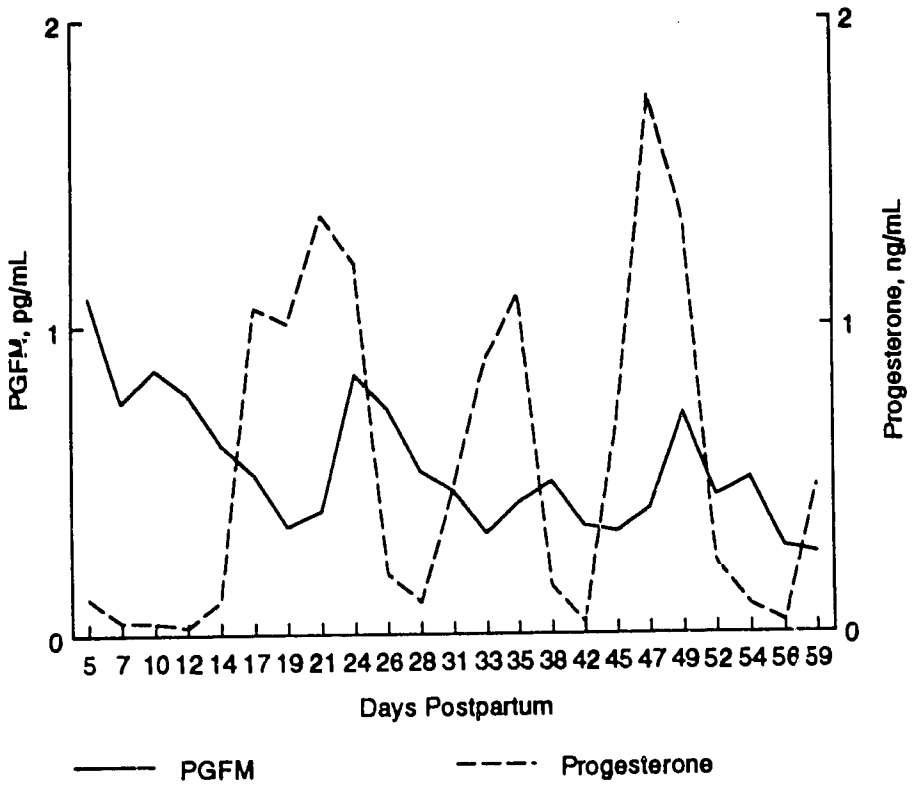
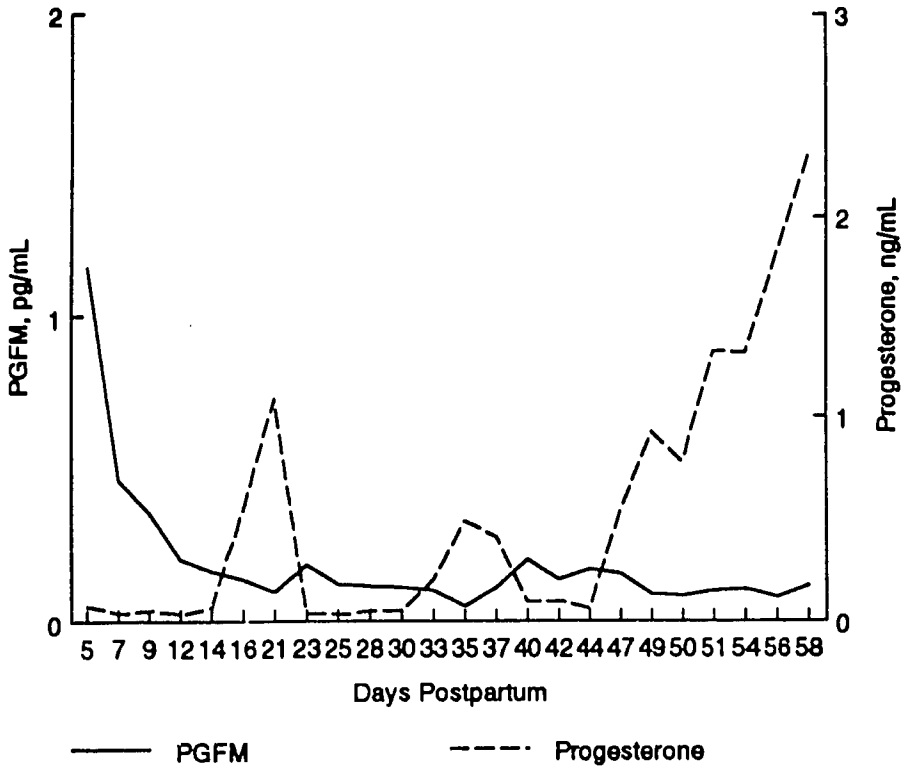


Cow # 415089

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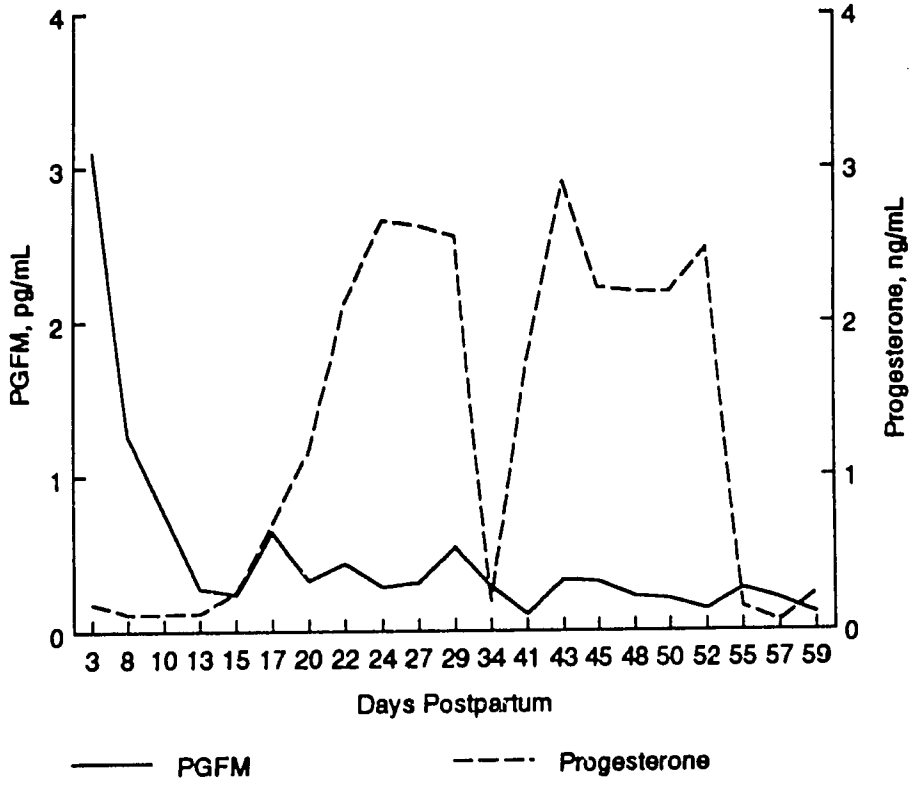

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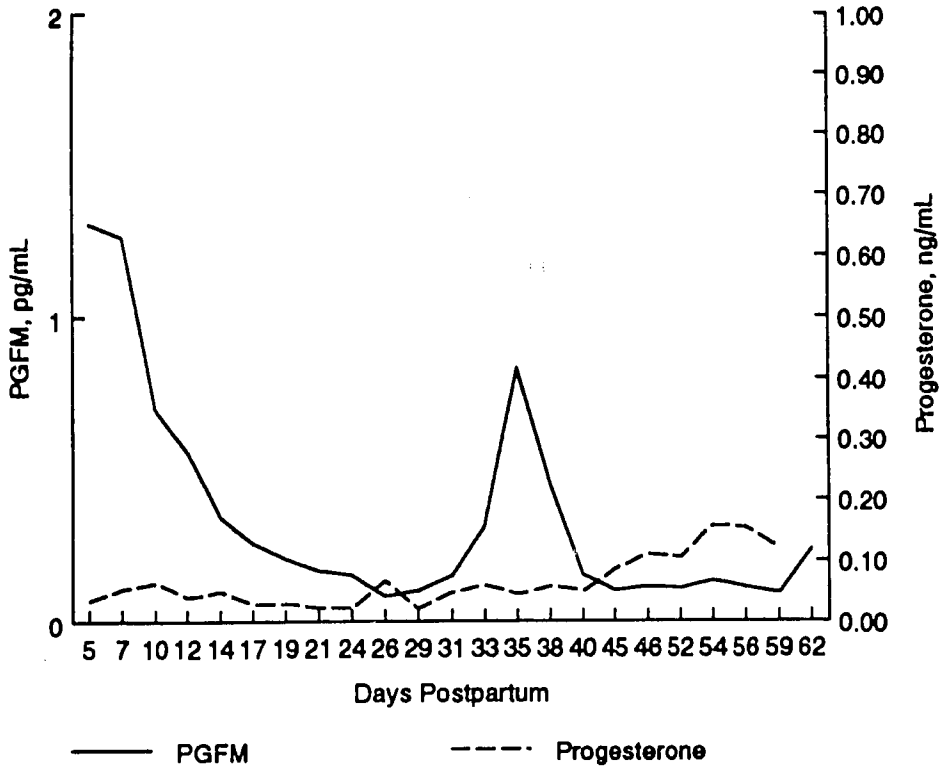
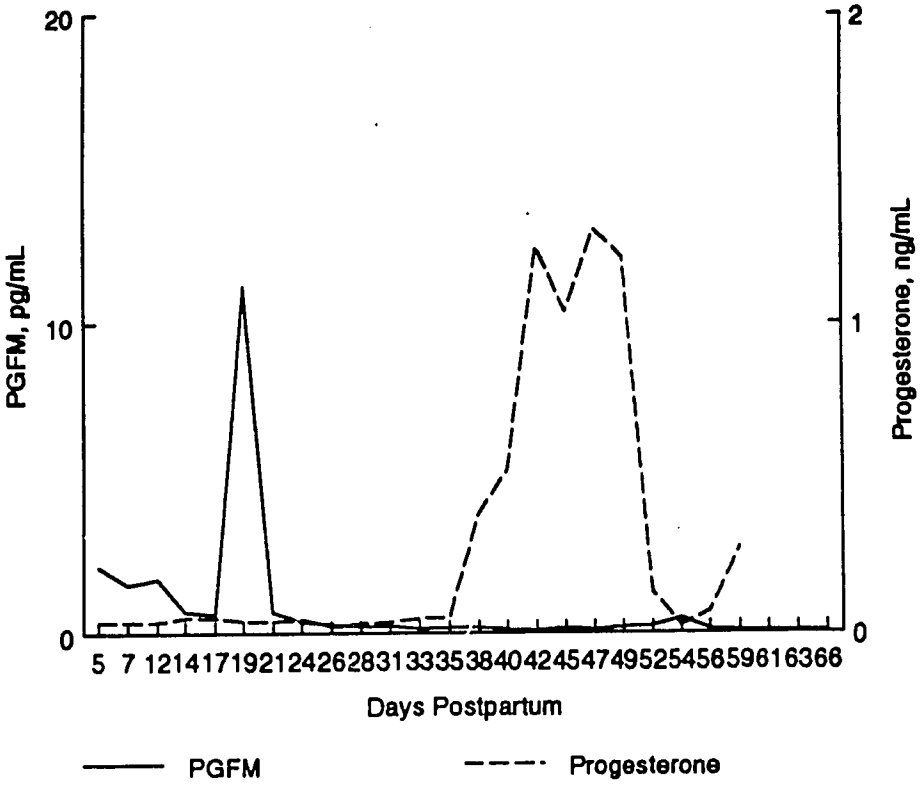



Cow # 713387



J Anim. Sci 69 Supp

# 1991 MEETING OF THE NORTHEAST ASAS

University of Maryland
College Park, Maryland
July 21–23, 1991

Material only within the blue line box will be photocopied exactly as received. Follow enclosed Guidelines for Abstract Submission for Members and the Criteria for Preparation of Abstracts.

Authors show choice of section in which they would like their paper scheduled:

- ☐ Graduate student competition
- ☒ Research
- ☐ Teaching
- ☐ Extension

Method of presentation

- ☐ Poster
- ☒ Oral


Corresponding author G. S. Lewis

Address Dept. of Animal Science
VPI&SU
Blacksburg, VA 24061-0306

Phone (703) 231-6797


EXAMPLE OF ABSTRACT HEADING AND ABSTRACT

Local and systemic effects of intramammary endotoxin infusion.
D. E. Shuster, R. J. Harmon, J. A. Jackson, and R. W. Hemken,
University of Kentucky, Lexington 40506.

To study the mechanisms responsible for the decline in milk production during mastitis, four cows were infused with 10 µg E. coli endotoxin in each of two homolateral quarters. Two other cows served as . . .

Abstract to be published by ASAS.

Visual Aid Preferred ☐ Overhead ☒ Slides

Induced endometritis elevates prostaglandin $F_2\alpha$ metabolite concentrations in postpartum beef cows. R.P. Del Vecchio, D.J. Matsas, T.J. Inzana and G.S. Lewis. Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0306.


Eighteen multiparous Angus cows were used to determine if the induction of endometritis would increase concentrations of 13,14-dihydro-15-keto-prostaglandin $F_2\alpha$ (PGFM) in jugular blood plasma. Beginning the day of calving (d=0), blood samples were collected on alternate days. On three consecutive days, ranging from d 8 to 14 of the first postpartum estrous cycle, uterine horns were inoculated transcervically with either 3 x $10^9$ colony forming units (CFU) Actinomyces pyogenes and 1.5 x $10^9$ CFU ß-hemolytic Escherichia coli (treated; n=9) in sterile phosphate-buffered saline (PBS) or with sterile PBS (control; n=9). Uterine fluid samples were collected transcervically twice weekly from just before the start of each series of inoculations until the end of the experiment. Endometrial biopsies were collected between d 4 to 6 and 11 to 13 after inoculation. Period 1 (PRD1) is the period before infusion. Period 2 (PRD2) is the period after infusion. Responders to bacterial infusion were cows with an increase in mean PGFM of at least 2 SD above their pre-treatment mean. Only responders (67%) were included in the PRD2 analysis of PGFM. Based upon clinical observations and bacterial cultures, all treated cows developed acute uterine infections. Controls did not develop uterine infections. Endometrial biopsies failed to show significant diffuse or focal cellular reactions in response to infection. Estrous cycle length was greater ($P<.0003$) in treated (27.7 ± 1.0 d) than in control cows (20.6 ± 1.0 d). In PRD1, mean PGFM concentration and PGFM profiles were similar ($P>.10$) between treated and control cows. In PRD2, treatment affected mean PGFM concentration ($P<.0001$) and PGFM profiles ($P<.06$). These data indicate that induced uterine infections increase jugular plasma concentrations of PGFM. Measuring plasma PGFM may be useful in early detection and treatment of subclinical endometritis.



KEY WORDS: Endometritis, Prostaglandin $F_2\alpha$ metabolite, Postpartum, Beef cows


Must be received by March 1, 1991, edited and ready for photocopy. Mail original etc. and 6 copies to L. E. Chase, Cornell University, 272 Morrison Hall, Ithaca, NY 14853-4801. If withdrawal of a paper becomes necessary, notify L. E. Chase (607-255-2196).

NOTE

The ASAS Abstract Form has been changed to accommodate the new size of JAS (8 1/2 x 11) starting January 1992. There will be four tracts on each page under new format.

IMPORTANT

Read all instructions before you type the abstract. Practice typing the abstract in a rectangle 13.5 x 17.5 cm before using the special form. Abstracts will be published as typed at 65% of original size. Use 10 point type or larger for abstract. Abstracts to be accepted, must be submitted on official abstract paper and in accordance with instructions including 'Quality Standards for Abstracts.'

INSTRUCTIONS

1. Material only within the blue line box will be photocopied exactly as received.
2. Arrange abstract in sample box before final typing on this form.
3. Correct mistake by affixing correction typed on white paper over error with rubber cement or use liquid paper.
4. Single space with no margins in the rectangle.
5. One line space between title and abstract.
6. Title without caps.
7. For multiple authors, designate presenter with * after name. Abbreviate only units of measure.

Authors show first (1), second (2), and third (3) choices:

- ☐ Awards Competition (specify second and third choice)
- ☐ Breeding and Genetics
- ☐ Extension
- ☐ Growth and Development
- ☐ Meat Science
- ☐ Nonruminant Nutrition
- ☐ Ruminant Nutrition
- ☐ Pastures and Forages
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- ☐ Teaching
- ☐ Withdraw paper if first section choice is not available

Signature of presenter

(504) 388-5419

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J. Anim. Sci. 70:(Suppl 1):23

# SOUTHERN SECTION AMERICAN SOCIETY OF ANIMAL SCIENCE

## ANNUAL MEETING
## 1992 ABSTRACT FORM
## February 1-5, 1992
## Lexington, KY

Original and four copies must be received by October 1, 1991 and should be edited, ready for the printer. All data must be reported in the metric system. If withdrawal of a paper becomes necessary, the Secretary should be notified at once.

Mail to: Paul Noland
Department of Animal Sciences
University of Arkansas
Fayetteville, AR 72701
(501/575-4549)

Effect of progestogen treatment on changes in plasma 13,14-dihydro-15-keto-prostaglandin $F_2\alpha$ in prepubertal beef heifers challenged with oxytocin. R.P. Del Vecchio*, E.E. Custer, G.S. Lewis and W.E. Beal. Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

Angus crossbred heifers (n=24) between 8 and 10 mo of age were used to determine if progestogen treatment affects prostaglandin $F_2\alpha$ ($PGF_2\alpha$) release in response to oxytocin (OT) by prepubertal bovine uteri. Heifers were stratified by age and weight and randomly allotted to treatments arranged in a 2 × 2 factorial. Heifers were treated with either a norgestomet (NOR) implant (6 mg) for 9 d or no implant (0 mg; BLK). On d 9 of NOR treatment and before implant removal, heifers were fitted with a jugular catheter, and blood samples were collected every 15 min for 165 min. The first four samples were used to determine basal 13,14-dihydro-15-keto-prostaglandin $F_2\alpha$ (PGFM) concentrations (an indirect measure of uterine $PGF_2\alpha$ release). After collection of the fourth sample, either OT (100 IU) or saline (0 IU; SAL) was injected via the jugular catheter. After the last sample was collected, NOR implants were removed. Beginning 48 h after implant removal, a second 165 min blood sampling period was conducted. Within treatment, PGFM concentrations were similar between the first and second bleeding period; therefore, data were combined. Basal PGFM concentrations were higher ($P<.01$) in NOR-treated heifers than BLK heifers. Oxytocin did not increase PGFM concentrations in the BLK-OT group. Mean PGFM was greatest ($P<.0001$) in the NOR-OT group and PGFM curves differed ($P<.0001$) between NOR-OT and all other treatments. Mean PGFM was greater ($P<.0001$) and PGFM curves differed ($P<.001$) between NOR-SAL and both BLK-OT and BLK-SAL groups. These data show that NOR increases basal PGFM and appears to "condition" the uterus to respond to OT in prepubertal heifers. Data from this study suggest that increases in progesterone measured 1 and 3 wk before first pubertal estrus may act to prepare the uterus for its role in the proposed positive feedback loop between luteal OT and uterine $PGF_2\alpha$.

**Key Words:** Prepubertal, Heifers, 13,14-dihydro-15-keto-prostaglandin $F_2\alpha$ Uterus, Oxytocin

EXAMPLE OF ABSTRACT HEADING AND ABSTRACT

Fertility of beef females following controlled estrus cycles and ovulation. A. A. Zaied*, W. D. Humphrey, C. C. Kaltenbach, and T. G. Dunn, University of Wyoming, Laramie.

Pregnancy rates (PR) following two progestogen implant periods and breeding at either controlled ovulation or 12 hr after synchronized estrus was compared...

List key words at end of abstract inside blue lines.

Journal of Animal Science Volume 70 Supplement 1 1992

Gregory S. Lewis

# 84th Annual Meeting
# Abstracts

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447 Effects of suckling and nutrition on endocrine profiles before and after estrus in postpartum Brahman cows. R. Browning, Jr *, A.W. Lewis, and R.D. Randel. Texas Agricultural Experiment Station, Overton.

Twenty-eight fall-calving Brahman cows were used to examine the effects of postpartum (PP) nutrition and controlled suckling on return to estrus and plasma concentrations of progesterone (P4) and 13,14-dihydro-15-keto-prostaglandin F2α (PGFM) before first estrus and P4 concentrations after estrus. At parturition (d 0 PP), each cow was randomly placed into 1 of 4 groups to receive either high or low nutrition and normal or once-daily suckling. Nutritional management began d 0 PP. Cows on the high (H) nutrition diet received 3.6 kg·hd-1·d-1 of a concentrate ration consisting of 3:1 corn:soybean meal. Low (L) nutrition cows received no concentrates. Coastal bermudagrass hay, water, and mineral supplement were available free choice. Suckling management began d 21 PP. Once-daily suckled (X) dams nursed their calves for 30 min each morning. Normal suckled (N) dams kept their calves at side. Analysis of plasma P4 and PGFM concentrations were made during the 10 d before first estrus. Plasma P4 concentrations were also analysed during the 18 d after estrus. The interval from calving to estrus was shorter in X females (42 vs 65 d; P<.01). The interval to estrus for HX, LX, HN, and LN dams were 37, 47, 67, and 62 d, respectively. Prior to first estrus, peak P4 concentrations for X and N dams were 1.68 and 1.26 ng/ml (P>.1), respectively and peak P4 concentrations for HX, LX, HN, and LN groups were 1.9, 1.46, 1.47, and 1.06 ng/ml (P>.1), respectively. Peak PGFM concentrations were higher in X dams (95 vs 25 pg/ml; P<.02) with peak PGFM concentrations before estrus measuring 136, 55, 26, and 23 pg/ml (P>.1) for groups HX, LX, HN, and LN, respectively. Further PGFM analysis was done based on cows having peak P4 concentrations >.7 ng/ml (S) or <.3 ng/ml (T) prior to estrus. Peak PGFM concentrations were higher in S cows (99 vs 20 pg/ml; P<.02). The corpora lutea formed by S cows secreted more P4 after estrus as determined by "area under the curve" (95.36 vs 13.23 area units; P<.01). Area under the post-estrus P4 curve for X and N dams were 70 and 67 area units (P>.1), respectively and 72, 68, 100, and 34 area units (P>.1) for HX, LX, HN, and LN groups, respectively. Secretion of P4 before and after estrus was correlated (r=.82). It is concluded that suckling can affect prostaglandin and P4 secretion before return to estrus and corpus luteum function following return to estrus in suckled beef cows.

**Key Words:** postpartum cows, management, endocrinology

448 Serum luteinizing hormone and cortisol concentrations du postpartum anestrus in Brahman and Angus cows. D. J. By R. C. Stahringer*, R. D. Randel and T. E. Kiser. Univers Georgia, Athens and Texas Agricultural Experiment St Overton.

Pluriparous suckled Brahman (B) and Angus (A) cows were utilized durin years (year 1:n=9/breed;year 2:n=10/breed) to compare hormonal pro during the postpartum (PP) anestrus. On d 17 and 34 PP, blood sam were collected at 15 min intervals for a 5 h period via jugular cannula that time, all animals received 10 ng/kg GnRH i.m. and were bled for an 2 h at the 15 min intervals. Serum was recovered and all samples assayed for LH by RIA. Hourly samples were assayed for cortisol by Mean serum LH (.63 ± .06 vs .96 ± .07 ng/ml), basal LH (.34 ± .03 vs .04 ng/ml) and area under the LH curve (177 ± 17 vs 292 ± 21 area [AU]) increased (P < .01) from d 17 to 34 PP. Height of the LH pulses ± .29 vs 2.13 ± .27 ng/ml) increased (P < .05) and amplitude of the LH p (1.0 ± .29 vs 1.61 ± .28 ng/ml) tended to increase (P < .1) by d 34 PP. frequency of the LH pulses was similar between days PP. Brahman cows higher (P < .05) mean LH (.89 ± .08 vs .70 ± .06 ng/ml), basal LH (.48 vs .36 ± .04 ng/ml), LH pulse frequency (2.2 ± .1 vs 1.5 ± .2 pulses/5 h area under the LH curve (261 ± 23 vs 208 ± 20 AU) than A cows. amplitude of the LH pulses (1.52 ± .34 vs 1.09 ± .21 ng/ml) tended (P to be greater in B than in A cows. The GnRH induced LH release was gre in B than in A cows (mean: 3.07 ± .31 vs 2.29 ± .22 ng/ml; pulse height: ± .63 vs 4.24 ± .36 ng/ml; area under the curve: 397 ± 39 vs 294 ± 26 Time to the LH peak (26.4 ± 1.5 vs 31.6 ± 2.3 min) tended (P < .07) shorter in B than in A cows. Mean serum cortisol concentrations (10.9 ± 8.6 ± .5 ng/ml) and area under the cortisol curve (1053 ± 68 vs 888 ± 54 decreased (P < .05) between d 17 and 34 PP. Mean serum co concentrations (8.6 ± .7 vs 10.9 ± .7 ng/ml) and area under the cortisol (855 ± 54 vs 1087 ± 65 AU) were lower (P < .01) in B than in A Secretion of LH increases while cortisol release decreases as PP p progresses. Under optimal environmental conditions B cows have a g LH release and their anterior hypophysis may be more sensitive physiological GnRH challenge compared with A cows.

**Key Words:** Luteinizing Hormone, Cortisol, Postpartum Anestrus

449 Prostaglandin $F_2\alpha$ metabolite concentrations in postpartum dairy cows with endometritis. R.P. Del Vecchio*, D.J. Matsas, S. Fortin, D.P. Sponenberg and G.S. Lewis. Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0306.

Postpartum Holstein (n=21) and Jersey (n=4) cows were used to determine whether plasma concentrations of 13,14-dihydro-15-keto-prostaglandin $F_2\alpha$ (PGFM) were higher in cows with endometritis than in herdmates without endometritis. Based on clinical examinations and bacterial content of intrauterine fluid samples, cows detected with endometritis between 21 and 28 d postpartum were used (infected; n=14). These cows were matched with herdmates that were free of infection (control; n=11). Beginning on the day cows were assigned to the experiment (d=0), blood samples were collected on alternate days for at least 14 d. Plasma samples were stored at -20° C until assayed. Uterine fluid samples were collected transcervically twice weekly, beginning on d 0, until the end of the experiment for aerobic bacterial culture. Endometrial biopsies were collected between d 6 to 8 and d 13 to 15. Control cows did not show signs of endometritis throughout the trial. Bacterial cultures showed no significant bacterial population present in the uterus of the control cows. The uterus of infected cows harbored numerous microbes, with Actinomyces pyogenes being most prominent. Escherichia coli and various species of Streptococcus and Pasteurella were also prevalent. Endometrial biopsies showed the infected cows had more neutrophils, plasma cells and lymphocytes in the endometrium than did control cows (P<.05). Plasma progesterone concentrations indicated that 83% of the control cows and 50% of the infected cows (P>.10) had functional luteal tissue, indicating ovarian activity, at some time during the 2 wk sampling period. Plasma PGFM curves were linear (P<.03) and did not differ between treatment groups (P>.10). Mean plasma PGFM concentrations were greater (P<.0001) in infected cows than in non-infected control cows. These data indicate that plasma PGFM concentrations are greater in postpartum cows with spontaneous endometritis than in herdmates without endometritis. Plasma PGFM may be used as an early indicator of uterine infections.

**Key Words:** Endometritis, Postpartum, Prostaglandin $F_2\alpha$ metabolite

450 Effect of RU 486 on induction of parturition in first-calf h Y. Li, B. Dlamini*, and L. L. Anderson, Iowa State Univ Ames.

RU 486 (11β-[4-dimethylamino phenyl]-17β-hydroxy-17α-[1-pro -estra-4, 9-dien-3-one, Roussel-UCLAF) is a 19-norsteroid with high for the progesterone receptor and it also has antiglucocorticoid acti multiparous beef cattle in late pregnancy, RU 486 precisely contro time of parturition without detrimental effects of dystocia, rete placenta, and delayed postpartum fertility (Endocrinology 129:7 1991). This study was designed to determine the effects of RU 4 or in combination with relaxin in late pregnant first-calf beef Twenty-one crossbred beef heifers were bred by artificial inseminat November 7-9, 1990 at the Chariton-McNay Cattle Research Cen day 265 (expected term = 283 d) the heifers were transferred to th Reproduction Laboratory for hormone treatments and calving. On the heifers were assigned randomly to three groups (n = 7 per grou 1 received RU 486 im at 2 mg/kg BW at 0800 h on d 277 and 27 2 received the same RU 486 treatment plus 3000 IU relaxin injec 0800 h on d 278; and group 3 served as controls and received dilu 70% ethanol; 3 ml PBS) by im injection on d 277 and 278. occurred 43 h after first treatment in group 1 and 52 h in group 2 with 182 h in the controls (P < 0.01). Birth weights of calves fr groups (29.1 and 27.3 kg in groups 1 and 2) were less than those calves (31.3 kg; P < 0.01). The incidence of difficult birth requiring manual assistance was 1 of 7 heifers in group 1 and 2 o in group 2 compared with 4 of 7 heifers in the control group. Th of delivery of placenta >24 h postpartum was 2 of 7 in group 1 in group 2 compared with 0 of 7 in the control group. C hormone-treated groups were vigorous at birth, and maternal be lactation of the heifers were normal. The results indica antiprogesterone, such as RU 486 or a potent analog, alone o with relaxin can be used to induce parturition in first-calf increase survival rates with smaller calves at birth and less i dystocia than seen in the diluent-treated control heifers.

**Key Words:** RU 486; Induced parturition; First-calf heifers

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# A QUANTITATIVE SOLID-PHASE ENZYMEIMMUNOASSAY FOR 13,14-DIHYDRO-15-KETO-PROSTAGLANDIN $F_{2\alpha}$ IN PLASMA[1]

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## ABSTRACT

Enzymeimmunoassays (EIA) can be viable alternatives to radioimmunoassays (RIA). Indeed, from an environmental perspective, EIA are preferable to RIA. Therefore, the purpose of this project was to develop a quantitative EIA for 13,14-dihydro-15-keto-prostaglandin $F_2\alpha$ (PGFM) in bovine plasma. Acetylcholine esterase bound covalently to PGFM, rabbit anti-PGFM, mouse monoclonal anti-rabbit IgG, and PGFM were the principle reagents used for the EIA. Validation experiments indicated that: 1) PGFM standard curves, with doses ranging from 391 to 200,000 fg per microtiter well, were linear; 2) assay sensitivity averaged 391 fg per well; 3) for satisfactory results, PGFM had to be extracted from plasma; 4) content of PGFM in ethyl ether extracts of aliquots from serial dilutions of whole plasma with unknown amounts of PGFM and charcoal-stripped plasma supplemented with known amounts of PGFM did not deviate from parallelism with PGFM standard curves in buffer; 5) correlation between EIA and RIA measurements of PGFM in the same plasma samples was .95; 6) the regression of EIA data on RIA data was linear ($Y = .93 X + 83.9$; $r^2 = .91$); 7) intra- and inter-assay coefficients of variation were 3.3 and 10.6 %, respectively. The EIA developed in this project is a valid and reliable method for quantitating PGFM in extracts of bovine plasma.

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## INTRODUCTION

Bioassays, chromatography, radioimmunoassays (RIA) and, most recently, enzymeimmunoassays (EIA) have been used to measure prostaglandins and other arachidonic acid metabolites. Methodological innovations have usually made prostaglandin assays more sensitive and simpler to perform. Thus, RIA has become, perhaps, the most popular method for measuring prostaglandins. Prostaglandin RIA are usually rapid, sensitive and simple, but RIA generate radioactive wastes that, because of environmental concerns and disposal costs, have become problems in many institutions. Enzymeimmunoassays have the analytical advantages of RIA, and EIA eliminate the problems associated with radioactive wastes.

Plasma concentrations of 13,14-dihydro-15-keto-prostaglandin $F_2\alpha$ (PGFM) are frequently measured in many physiological, endocrinological and clinical studies. In the bovine, measuring blood plasma PGFM has been used by numerous investigators to monitor uterine prostaglandin $F_2\alpha$ production in postpartum cows (1,2,3). Significant correlations have been found between the duration of elevated PGFM and the time required for complete uterine involution as well as the interval from parturition to first ovulation followed by a normal luteal phase (4).

The purpose of this project was to develop a sensitive, simple, quantitative EIA for PGFM in bovine plasma. Enzymeimmunoassays for PGFM have been reported (5,6), but those PGFM EIA did not have the combination of sensitivity and simplicity that we believe is desirable for routine use.

## MATERIALS AND METHODS

### EIA reagents.

Acetylcholine esterase (AChe; EC 3.1.1.7, molecular form = G4) from electric eels (Electrophorus electricus) was bound covalently to PGFM (3), and the PGFM-AChe conjugate (Cayman Chemical Co.; Ann Arbor, Michigan) was used as EIA tracer. Acetylcholine esterase was chosen because previous work indicated that the combination of sensitivity and simplicity of AChe-based EIA was superior to that for EIA developed with other PGFM-enzyme conjugates (5,6,7). Other reagents used for the EIA were: rabbit anti-PGFM (WS 4468-4-1) from Dr. W.J. Silvia (University of Kentucky); mouse monoclonal anti-rabbit IgG (MAB; Cayman Chemical Co.); Ellman's reagent (750 μM 5,5'-dithio-bis(2-nitrobenzoic acid) and 500 μM acetyl thiocholine iodide; Cayman Chemical

Co.); authentic PGFM for standards (Sigma Chemical Co.; St Louis, Missouri). The PGFM-AChe conjugate, MAB and Ellman's reagent were stored lyophilized until just before they were used. Rabbit anti-PGFM was received lyophilized. Crossreactivity of anti-PGFM lot WS 4468-4-1 was identical to that for WS 4468-3 (W.J. Silvia, personal communication), which was < .1% with $PGF_2\alpha$, $PGE_2$, $PGA_2$ and 6-keto-$PGF_1\alpha$ (8).

EIA buffers.

Four primary buffers were used for this EIA; they were potassium phosphate buffer (1 M), EIA buffer, saturation buffer and wash buffer. All buffers were prepared with deionized, organic-free, reagent-grade water. Potassium phosphate buffer (1 M) contained 174.18 g of potassium phosphate, dibasic and 136.09 g of potassium phosphate, monobasic per liter, and 10 M potassium hydroxide was used to adjust pH to 7.4. Potassium phosphate buffer (1 M) was used to prepare other buffers. A liter of EIA buffer contained 23.4 g sodium chloride, 370 mg tetrasodium EDTA, 1 g fraction V bovine serum albumin (BSA; 98 to 99 % albumin; Sigma Chemical Co.), 100 mg sodium azide and 100 ml of 1 M potassium phosphate buffer; pH was adjusted to 7.4 with 10 M potassium hydroxide. Saturation buffer was the same as EIA buffer, except saturation buffer contained 3 g BSA and 300 mg sodium azide per liter. A liter of wash buffer contained 10 ml of 1 M potassium phosphate buffer and .5 ml Tween 20 (Sigma Chemical Co.). All buffers were stored at 4°C.

Reagent dilutions.

The PGFM-AChe in each vial from the supplier was reconstituted with 60 ml of EIA buffer; PGFM-AChe was packaged as 500 determinations per vial. Aliquots (6 ml) of PGFM-AChe solution (master dilution) were stored at -20°C. For use in the EIA, aliquots of the master dilution of PGFM-AChe were diluted with EIA buffer (2:1 PGFM-AChe solution:EIA buffer). Rabbit anti-PGFM was reconstituted with water, diluted 1:100 with EIA buffer, and stored at -20°C in 1 ml aliquots. For use in the EIA, aliquots of anti-PGFM were thawed and diluted to 1:800,000 with EIA buffer. Mouse monoclonal anti-rabbit IgG in each vial from the supplier was reconstituted with 100 ml of .05 M potassium phosphate buffer; MAB was packaged as 500 determinations (1000 μg) per vial. Ellman's reagent (250 determinations per vial) was reconstituted with 50 ml of water. Authentic PGFM was diluted with 100 % ethanol to a concentration of 10 ng/ml (master dilution) and stored at -20°C until it was used to prepare EIA standards. Ethanol



was evaporated from aliquots of the master dilution of PGFM, and assay standards in doubling doses from 391 to 200,000 fg/50 μl were prepared in EIA buffer. This allowed for the measurement of 7.8 to 4000 pg/ml using a 50 μl sample volume.

PGFM extraction.

Because initial results indicated that whole blood plasma interfered with the EIA, PGFM was extracted from plasma. Anhydrous ethyl ether was used to extract PGFM from acidified (25 μl of 1 N HCl/200 μl plasma) bovine plasma (200 μl). The ratio of ether to plasma was 15:1. Freezing was used to separate ether and aqueous phases. Ether phases were decanted into polypropylene tubes, and the ether was evaporated at room temperature with a stream of air. In previous work with RIA and high-performance liquid chromatography, we found that air did not damage PGFM appreciably; however, evaporating solvents with air, rather than nitrogen, damaged $PGE_2$ and $PGF_2\alpha$. Ether extracts were reconstituted with 200 μl of EIA buffer. Efficiency of extracting [$^3$H]PGFM from bovine plasma averaged 91%.

Equipment.

Essential equipment for the EIA included: polystyrene microtiter plates and self-adhesive polyester sealing film (Nunc Maxisorb type II-F96; Thomas Scientific, Swedesboro, New Jersey); microtiter plate spectrophotometer (Titertek Multiskan; Flow Laboratories Inc., McLean, Virginia); water purification system (Corning Mega-pure MP-190 LC, Corning, New York); orbital shaker.

EIA procedures.

To coat wells in microtiter plates, 200 μl of MAB solution (10 μg/ml) were pipetted into each well; MAB binds to sites on the surface of the wells. Plates were covered with sealing film and incubated for 18 to 20 hr at room temperature. To block binding sites remaining on the surface of the wells after incubation with MAB, 100 μl of saturation buffer were added to MAB solution in each well. Plates were covered with sealing film and incubated at 4°C. After at least 18 hr at 4°C, plates could be used immediately for EIA, or they could be stored for several weeks at 4°C. Plates were stored with MAB and saturation buffer in the wells.

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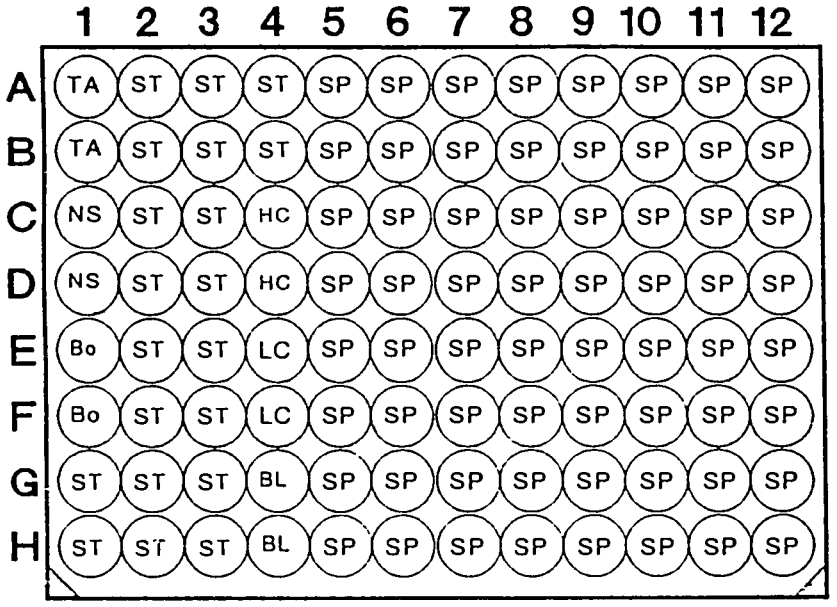


Figure 1. Outline of a microtiter plate showing the arrangement of total activity (TA), non-specific binding (NS), total binding ($B_o$), PGFM standard (ST), high (HC) and low (LC) PGFM concentration control samples, solvent blank (BL) and sample (SP) wells.

For EIA, microtiter plates were warmed to room temperature, and solutions were emptied from the wells. Wells were rinsed three times with wash buffer. Wash buffer was allowed to drain from the plates for about 5 min after the last rinse.

Standards and reconstituted extracts from plasma samples were pipetted (50 µl) into microtiter wells; the format is depicted in Figure 1. Initial trials indicated that position in the plate did not affect standard curves. So for convenience, standard curves were positioned at the front of each plate. Except for total activity (TA) wells, PGFM-AChe (50 µl) solution was added to each well. Except for TA and nonspecific binding (NSB) wells, anti-PGFM (50 µl) was added to each well. EIA buffer was used to adjust NSB and total binding ($B_o$; PGFM-AChe and anti-PGFM only) wells to 150 µl. Plates were incubated at room temperature.



After 18 to 20 hr of incubation, solutions were emptied from wells, and wells were rinsed five times with wash buffer. PGFM-AChe (5 µl) was added to TA wells, and 200 µl of Ellman's reagent were added to all wells. Plates were covered with sealing film and placed in the dark on an orbital shaker. Optical density (OD; measured at 414 nm) of solutions in microtiter wells was measured periodically. When OD for $B_o$ wells was between .3 and .5 units, which was about 6 h after addition of Ellman's reagent, OD was measured in all wells in a plate.

Because the volume of PGFM-AChe added to TA wells was 10% of that added to other wells, OD for TA wells was multiplied by 10. Adjusted OD for TA wells was used to calculate percent binding. Equations derived from the regression of logit of percent binding on log of concentration were used to calculate the amount of PGFM in extracts of plasma. Content of PGFM per well was not corrected for extraction efficiency.

PGFM radioimmunoassay.

Procedural details and validation statistics for the RIA used in this project have been reported (3,9).

EIA validation procedures.

The Statistical Analysis System (SAS; 10) was used for all data analyses. Regression analysis was used to derive the equation that best described the shape (i.e., linear, quadratic, etc.) of standard curves. To determine if plasma PGFM curves and standard PGFM curves in EIA buffer deviated from parallelism, bovine plasma samples (n=15) with unknown amounts of PGFM and charcoal-stripped bovine plasma (CSP) supplemented with known amounts of PGFM (n=5 lots) were diluted serially with CSP. PGFM was measured in extracts from aliquots of diluted plasma. Analyses of variance and tests for heterogeneity of residual variances were used to determine if plasma PGFM curves and standard PGFM curves deviated from parallelism.

Enzymeimmunoassay and RIA were used to measure PGFM in 40 bovine plasma samples in which PGFM concentrations were expected to range from low to high. Analysis of variance was used to determine if PGFM concentrations differed between EIA and RIA. Pearson product-moment correlation was used to determine the degree of association between EIA and RIA data. The regression of EIA data on RIA data was used to derive the equation that best described the mathematical relationship between PGFM measurements made with the two

analytical procedures. Regression equations usually indicate cause and effect relationships. However, the regression equation derived from this analysis of EIA and RIA data is only descriptive and not indicative of a cause and effect relationship.

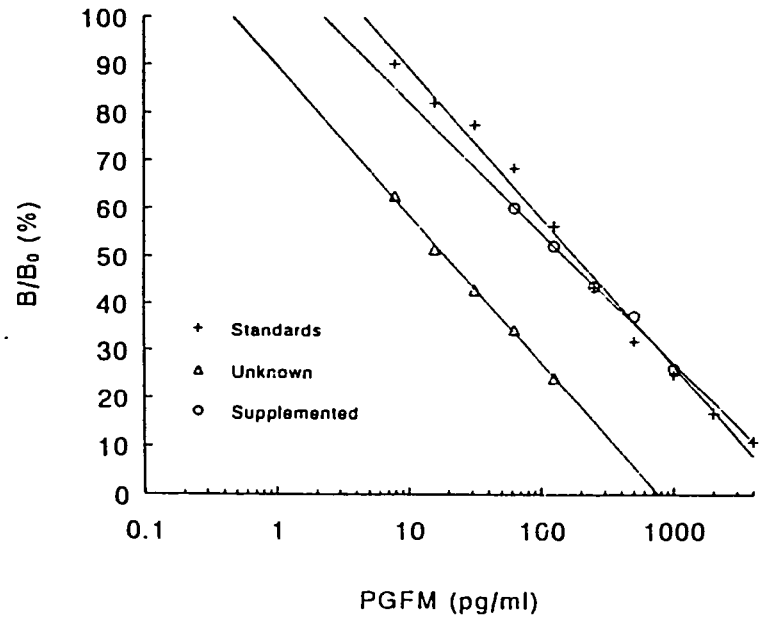


Figure 2. Relationship between plasma PGFM curves and standard PGFM curves in buffer. Charcoal-stripped bovine plasma (CSP) supplemented with known amounts of PGFM and bovine plasma samples with unknown amounts of PGFM were diluted serially with CSP, and PGFM was measured in extracts of aliquots of diluted plasma. Curves did not deviate (P > .10) from parallelism.



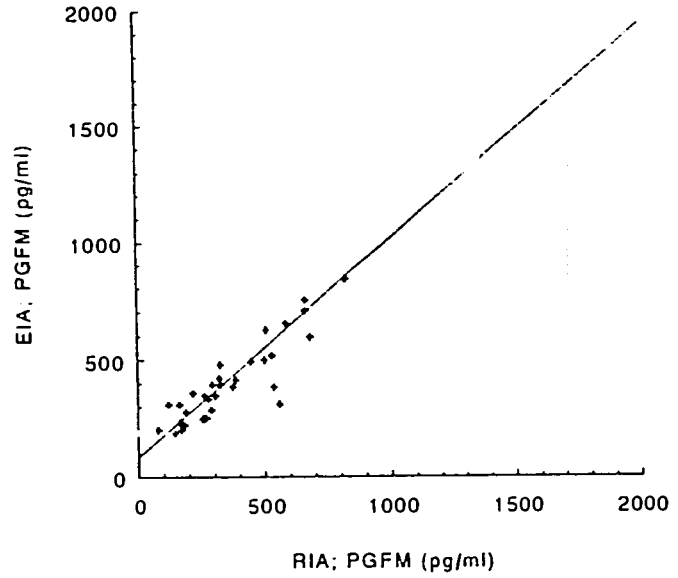


Figure 3. Relationship between concentrations of PGFM measured with enzymeimmunoassay (EIA) and radioimmunoassay (RIA) in extracts of the same bovine plasma samples (n = 40). The equation, Y = .93 X + 83.9 ($r^2$ = .91), best described the mathematical relationship between EIA and RIA data. Correlation (.95) between EIA and RIA data was significant (P < .0001).

**RESULTS**

Standard curves were linear over doses ranging from 391 to 200,000 fg of PGFM per microtiter well. At 93 % relative binding (calculated from 23 assays), EIA sensitivity was 391 fg/well, which was equivalent to 7.8 pg/ml plasma.

Plasma PGFM curves and standard PGFM curves in buffer did not deviate (P > .10) from parallelism (Figure 2). Concentrations of PGFM measured with EIA did not differ (P > .10) from those measured with RIA. The correlation between EIA and RIA data was .95 (P < .0001). The equation, Y = .93 X + 83.9 ($r^2$ = .91), best described the mathematical relationship between EIA and RIA measures of PGFM in

extracts of the same plasma samples (Figure 3). Based upon data from extracts of control plasma samples included in 23 assays, intra- and inter-assay coefficients of variation were 3.3 and 10.6 %, respectively.

**DISCUSSION**

Results indicate that the EIA developed in this project is a valid and reliable method for quantitating PGFM in extracts of bovine plasma. Our EIA is considerably more sensitive (391 fg/well) than is our RIA (10,000 fg/tube) for PGFM or the RIA developed with the same PGFM antibody used in our EIA (77,000 fg/ml; 8). In addition, our EIA can be completed more rapidly and is inherently more environmentally responsible than is our RIA. Replacing sodium azide (LD 50 = 27 mg/kg in rats; 11) in buffers with another less toxic preservative such as thimerosal (LD 50 = 98 mg/kg in rats; 12), which should not affect assay validity, could enhance the overall safety of our EIA. Reagent costs per plasma sample for the EIA and RIA are comparable, but our estimates did not include the cost of disposing of radioactive wastes from the RIA.

Based upon the literature, our assay for PGFM is more sensitive than one EIA, which used a ß-galactosidase-PGFM conjugate (391 vs. 3,540 fg/well; 5), and less sensitive than another, which used a biotin-streptavidin amplified peroxidase system (391 vs. 57 fg/well; 6). The PGFM EIA with the biotin-streptavidin amplified peroxidase system appears to be more complicated and less rapid than our assay. Thus, we believe that our EIA combines the sensitivity and simplicity needed for making routine measurements of PGFM in large numbers of bovine plasma samples.

**ACKNOWLEDGEMENTS**

The authors thank Lee Johnson and Jim McDonald for their invaluable technical assistance throughout this project.

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# Effect of Intrauterine Bacterial Infusions and Subsequent Endometritis on Prostaglandin $F_{2\alpha}$ Metabolite Concentrations in Postpartum Beef Cows[1]

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ABSTRACT: Multiparous Angus and crossbred Angus cows were used to determine the effect of induced endometritis on plasma concentrations of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) and progesterone ($P_4$) and on duration of the estrous cycle of treatment. Beginning on the day of calving (d 0), blood samples were collected on alternate days. On three consecutive days, ranging from d 8 to 14 of the first postpartum estrous cycle, uterine horns were inoculated transcervically with either $3 \times 10^9$ colony forming units (cfu) of *Actinomyces pyogenes* and $1.5 \times 10^9$ cfu of β-hemolytic *Escherichia coli* (treated; n = 9) in sterile PBS or with sterile PBS alone (control; n = 9). Samples of uterine fluid were collected by transcervical aspiration twice weekly from just before the start of each series of inoculations until the end of the experiment. Endometrial biopsies were collected transcervically between d 4 to 6 and 11 to 13 after inoculation. Based on clinical observations and results of bacterial cultures, all treated cows developed acute uterine infections. Controls did not develop uterine infections. Endometrial biopsies indicated that there were no significant diffuse or focal cellular reactions in response to the infection. The interestrous interval was greater ($P < .0003$) for treated (27.7 ± 1.0 d) than for control (20.6 ± 1.0 d) cows, but $P_4$ concentrations were similar between the two groups. Mean PGFM concentration and PGFM profiles were similar ($P > .10$) between treated and control cows before bacterial infusions. Bacterial infusions increased mean PGFM concentration ($P < .0001$) and changed the shape of the PGFM profile ($P < .02$). These data indicate that induced uterine infections increase peripheral plasma concentrations of PGFM and extend the interestrous interval. They demonstrate the potential for using measurements of PGFM as an indicator of uterine infection in cattle.

Key Words: Endometritis, Prostaglandins, Postpartum Interval, Bovidae

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## Introduction

In female livestock, reproductive efficiency is the major determinant of lifetime productivity. Reproductive disorders are a major reason for decreased reproductive efficiency in cattle. Endometritis is a common reproductive disorder, accounting for about 20% of the reproductive disorders in dairy cattle (Coleman et al., 1985). As many as 67% of the postpartum cattle examined in one study had endometritis (Ruder et al., 1981). Consequences of endometritis in cattle can range from no effect on reproductive performance to permanent sterility (Griffin et al., 1974). Endometritis increases intervals from calving to ovulation, detected estrus, and insemination and conception, and its delays uterine and cervical involution (Lindell et al., 1982; Fonseca et al., 1983; Coleman et al., 1985).

Both aerobic and anaerobic bacteria have been cultured from uteri of cattle with endometritis (Griffin et al., 1974; Peter and Bosu, 1987). When uteri were harboring *Actinomyces pyogenes* after about d 21 postpartum, the cows developed severe

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endometritis and were almost invariably infertile at the first service (Griffin et al., 1974). *Actinomyces pyogenes* seems to be the bacterium consistently associated with severe endometritis.

There seems to be a relationship between endometritis and concentrations of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) in peripheral blood plasma. However, this relationship has been described in only a small number of cows, and these cows developed endometritis spontaneously (Lindell et al., 1982; Peter and Bosu, 1987). Changes in PGFM after calving reflect the rate of uterine and cervical involution (Lindell et al., 1982; Lewis et al., 1984; Guilbault et al., 1987). In general, cyclic ovarian function in postpartum cattle usually does not resume until concentrations of PGFM are basal (Eley et al., 1981; Lewis et al., 1984; Madej et al., 1984). Endometritis lengthens the interval from calving to return to basal PGFM (Lindell et al., 1982). Even though there seems to be a relationship between endometritis and PGFM in peripheral plasma, the effects of endometritis on PGFM concentrations have not been tested experimentally. Therefore, the objectives of this study were to 1) induce an acute uterine infection in postpartum cows and 2) determine the effect of induced endometritis on the interestrous interval and jugular plasma concentrations of PGFM and progesterone ($P_4$).

## Materials and Methods

Multiparous Angus and crossbred Angus cows that calved between September 22 and November 5, 1989, were allotted randomly by calving date to either a control or bacterial-infusion group. All cows were observed daily from approximately 0700 to 0730 and again from 1530 to 1600 for visual signs of estrus. Estrus was determined to have occurred if a cow stood for mounting. On three consecutive days, ranging from d 8 to 14 after first postpartum estrus, both uterine horns in each cow were inoculated transcervically with 30 mL of sterile PBS containing $3 \times 10^9$ colony forming units (cfu) of *A. pyogenes* and $1.5 \times 10^9$ cfu of β-hemolytic *Escherichia coli* (n = 9 cows; *E. coli* has been used for many years to induce acute uterine inflammations; Brinsfield et al., 1964; Hawk et al., 1964) or with 30 mL of PBS only (n = 9 cows). Infusion pipettes sterilized with ethylene oxide and covered with an outer sheath to prevent vulval and vaginal contamination were used to make transcervical infusions. Bacteria used in the inocula throughout the experiment were obtained from a cow with severe endometritis in the Virginia Polytechnic Institute and State University dairy herd. The bacteria were cultured at the Virginia-Maryland Regional College of Veterinary Medicine Clinical Microbiology laboratory, where they were grown on Columbia Blood Agar (CBA) plates, isolated, identified, and stored in frozen culture (70% sterile skim milk) until they were used to prepare each inoculation. Bacteria from frozen culture were streaked onto agar plates (CBA for *A. pyogenes* and Brain Heart Infusion (BHI) agar for *E. coli*) and allowed to grow until sufficient colony formation (16 to 48 h) was detected. Colonies were transferred with a sterile calcium alginate swab into sidearm flasks containing 15 to 20 mL of BHI broth for *E. coli* or BHI broth supplemented with 10% horse serum for *A. pyogenes*. Bacteria were grown at 37°C in a shaker water bath at 120 rpm until turbid. After it became turbid, the broth was decanted into a sterile 30-mL Corex centrifuge tube and spun at 12,000 × *g* for 10 min. The supernatant was decanted, and the pellet was resuspended in sterile PBS and centrifuged again; this step was repeated for a total of two washings in PBS. The resultant pellet was resuspended and diluted in sterile PBS to a concentration of $1 \times 10^9$ cfu/mL. Bacterial concentration was determined by spectrophotometry and confirmed by a viable plate count. Bacteria were further diluted with PBS to $2 \times 10^8$ cfu/mL for *A.pyogenes* and $1 \times 10^8$ cfu/mL for *E. coli*. A 15-mL volume of each bacterial solution was transferred into a sterile 60-mL syringe; the syringe contained a total of $3 \times 10^9$ cfu of *A. pyogenes* and $1.5 \times 10^9$ cfu of *E. coli* and was used to inoculate the uteri in each treated cow.

Beginning on the day of calving (d 0) and continuing until the end of the experiment, jugular blood samples were collected on alternate days. Within 45 min after collection, the samples were centrifuged at 1,000 × *g* for 25 min. Plasma was collected and stored at −20°C until it was assayed for PGFM using enzymeimmunoassay (EIA) procedures described by Del Vecchio et al. (1992) and for $P_4$ using RIA procedures described by Harrison et al. (1985). The inter- and intraassay CV for PGFM and $P_4$ were 10.6, 3.3, 8.3, and 6.9%, respectively.

Using sterile infusion pipettes with an attached syringe, uterine luminal fluid samples were collected transcervically twice weekly from just before the start of each series of inoculations until the end of the experiment. Each sample was transferred immediately into a sterile glass vial, capped, and transported to the clinical microbiology laboratory for bacterial culture and subsequent identification of microorganisms. Endometrial biopsies were collected transcervically between d 4 to 6 and 11 to 13 after the last inoculation. Biopsy specimens were placed immediately into Modified Bouin's fixative (73.1% picric acid, 24.4% formalin, 2.5% glacial acetic acid). After a 24- to 36-h fixation period, endometrial biopsies were transferred to 70%

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ethanol and stored until they were prepared for histological evaluation (Griffin et al., 1974).

At approximately 14 to 16 d after the last inoculation, cows were treated with prostaglandin $F_{2\alpha}$ (25 mg i.m.; Lutalyse, Upjohn, Kalamazoo, MI) to help clear the uterus of infection. After the cows were considered free of infection, as determined by clinical observations and bacterial cultures, they were returned to the breeding herd (approximately 8 to 10 d after prostaglandin $F_{2\alpha}$ treatment).

All data were analyzed with the GLM procedure of SAS (1985). Split-plot analyses of variance with day as a continuous independent variable and tests for homogeneity of residual variances were used to determine the effect of treatment on changes in concentrations of PGFM over time during the experiment. Polynomial regression analysis was used to determine the shape of PGFM curves. Separate analyses of variance were used to determine the effect of treatment on cycle length and concentrations of $P_4$. For the purposes of this study estrous cycle length was calculated from the first postpartum estrus to the next estrus and ovarian cycles were determined by concentrations of $P_4$ in peripheral blood plasma.

## Results

Mean number of days from parturition to first estrus were similar ($P > .10$) between the treated (52.3 ± 21.3 d) and the control groups (48.4 ± 17.9 d). Cultures of uterine aspirates indicated that uteri from all cows were free of pathogenic microorganisms during the preinfusion period. Based on bacterial cultures and the presence of cloudy vaginal discharge, uterine infections were established in cows that were infused with bacteria. Uterine fluid samples from all nine cows infused with bacteria contained *A. pyogenes* and β-hemolytic *E. coli* during the postinfusion period. Cows infused with sterile PBS did not develop uterine infections, and fluid samples from these cows did not contain any bacterial pathogens.

Endometrial biopsies were similar between control and bacterial-infused cows. Biopsies collected from the endometritic cows indicated that there were no significant diffuse or focal cellular endometrial reactions in response to the bacterial infusions. Therefore, the induced uterine infections were classified as mild and acute. The induction of endometritis did not affect first-service conception rate, services per conception, or pregnancy rate in this study (Table 1).

Progesterone concentrations were similar ($P > .10$) between the two groups of cows during the postinfusion period (Figure 1). Based on detection of estrus, induced endometritis increased ($P < .0003$) the interestrous interval from 20.6 ± 1.0 d for the control cows to 27.7 ± 1.0 d for the cows infused with bacteria. At the end of the experiment (i.e., 14 to 16 d after the last inoculation) all endometritic cows received prostaglandin $F_{2\alpha}$, which induced estrus. It is uncertain how long the interestrous interval would have been extended if the prostaglandin $F_{2\alpha}$ treatment had not been administered.

Table 1. First-service conception rate, services per conception, and pregnancy rate of the control and treated cows

| Group | First-service conception | Services per conception | Pregnancy rate |
|---|---|---|---|
| Treated | 66.7% (6/9) | 1.50 | 100% (9/9) |
| Control | 66.7% (6/9) | 1.56 | 77.8% (7/9) |

Mean PGFM concentration and PGFM profiles were similar ($P > .10$) during the preinfusion period between control and bacterially infused cows (Figure 2). During the postinfusion period, bacterial infusion treatment with subsequent endometritis increased ($P < .0001$) mean PGFM concentration (200.1 ± 5.1 vs 323.0 ± 32.2 pg/mL for the control and treated cows, respectively) and changed the shape of the PGFM profile of the treated cows ($P < .02$) compared with that of the control cows (Figure 3).

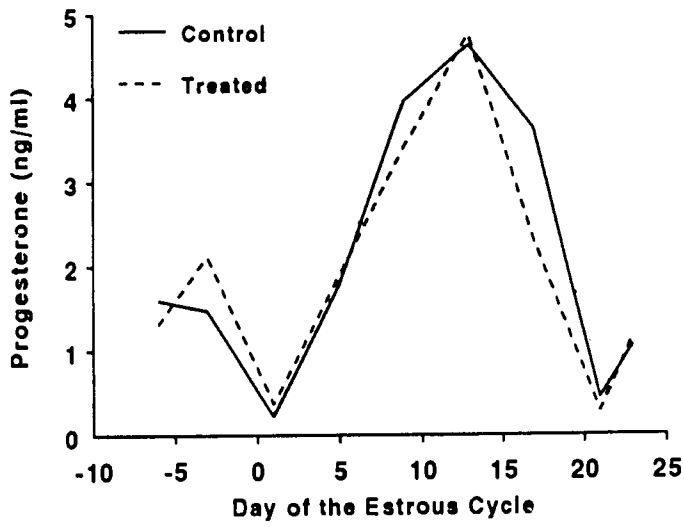


Figure 1. Mean plasma progesterone ($P_4$) concentrations of control (saline infused) and treated (bacterial infused) cows from 5 d before the first postpartum estrus (d 0) through 23 d after first estrus. Intrauterine infusions were given between d 8 and 14 of the estrous cycle. Concentrations of $P_4$ were similar between the two groups of cows.

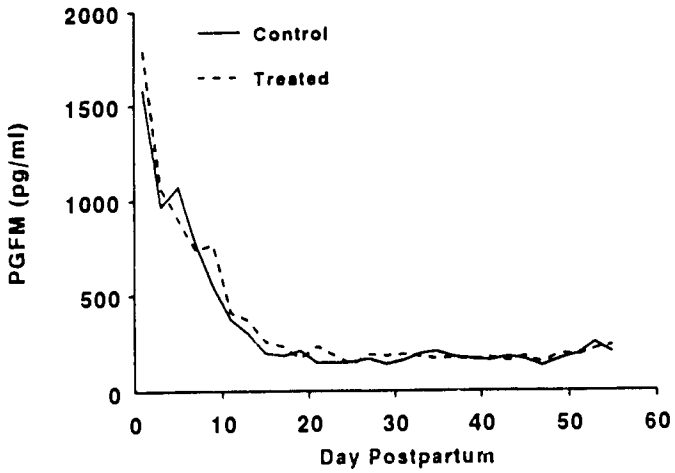


Figure 2. Mean plasma 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) concentrations of control (saline infused) and treated (bacterial infused) cows during the early postpartum period, before the intrauterine infusions. Concentrations of PGFM were similar between the two groups of cows.

## Discussion

These data indicate that the intrauterine infusion of bacteria described in the present study induces a mild, acute endometritic condition in postpartum cows. In previous publications on cattle and sheep, procedures to produce acute uterine infections with intrauterine infusions of *E. coli* were described (Brinsfield et al., 1964; Hawk et al., 1964). The inclusion of *A. pyogenes*, the most prevalent aerobic bacterium found in the uterus of endometritic cows, with *E. coli* in our inocula increased the likelihood of inducing uterine infections. El-Azab et al. (1988) and Farin et al. (1989) induced endometritis in postpartum cattle with intrauterine infusions of *A. pyogenes* and Gram-negative anaerobic bacteria (*Bacteroides melaninogenicus* and *Fusobacterium necrophorum*). The procedural model described in the present study used only aerobic microorganisms. These organisms have less stringent culture and collection requirements than do anaerobic organisms, which simplifies the preparation of the inocula and handling of bacterial cultures.

Concentrations of progesterone after intrauterine infusions revealed that the cows in both groups continued to have ovarian cycles. However, cows in the bacterially-infused group failed to exhibit estrus. Previous studies with cows indicate that uterine infections can cause the demise of the corpus luteum (CL and increases in PGFM concentrations (Gallagher and Ball, 1980; Peter and Bosu, 1987). When a uterine infection develops into pyometritis, the CL can become persistent, as determined by palpation, and the cows can become acyclic (Farin et al., 1989; Vighio et al., 1991). The induced uterine infections in the present study did not develop into pyometra and the CL of the cows were not persistent, but the cows became anestrous. The behavioral modification apparently caused by the induction of endometritis in this study may be mediated through elevated endotoxin levels in the blood. Increases in blood endotoxin levels have been shown to elevate endogenous opioid concentrations (Carr et al., 1982) that inhibit pituitary gonadotropin release (Malven, 1986; Haynes et al., 1989; Barb et al., 1991). As a result, changes in follicular development may have occurred, resulting in modified behavior.

Results of this study indicate that bacterial infusions increased average jugular plasma PGFM concentrations and changed PGFM profiles in postpartum cows. Increases in PGFM concentrations after first postpartum ovulation have been associated with moderate to heavy intrauterine bacterial growth patterns and infection (Peter and Bosu, 1987). Postpartum cows with pyometra or endometritis had higher basal PGFM concentrations than did their herdmates without an infection (Watson, 1984; Mann et al., 1985). In contrast, Vighio et al. (1991) reported that cows with pyometra had PGFM concentrations that were similar to those in noninfected controls. Studies in

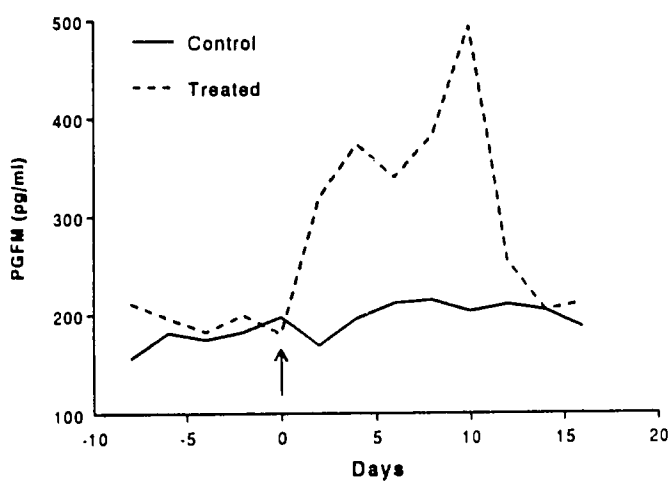


Figure 3. Mean plasma 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) concentrations of control (saline infused) and treated (bacterial infused) cows from 8 d before the intrauterine infusions through 16 d after infusions. The arrow indicates the average day of the first intrauterine infusion. Mean PGFM concentrations were greater ($P < .0001$) in the infected than in the control cows and the shape of the PGFM profiles ($P < .02$) differed between the two groups of cows.

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which PGFM was measured in postpartum cows with uterine infections have been mainly conducted with cows that developed the infection spontaneously. Peter et al. (1990) infused *E. coli* endotoxin into the uterus of cows and experimentally induced an increase in blood plasma PGFM concentrations on d 5 postpartum. However, because these researchers did not infuse bacteria or induce an infection in their cows, the rise in PGFM was short-lived, only 4 h on d 5 and not detectable when the cows were infused on d 20. With the exception of the present study and that of Peter et al. (1990), only a limited amount of data is available relating systemic PGFM concentrations with the development of endometritis in postpartum cows to study the endocrinological aspects of the uterus in response to the infection.

## Implications

By studying uterine responses to the induction of endometritis, we have shown a distinct biochemical response. Given the clear relationship between jugular plasma prostaglandin $F_{2\alpha}$ metabolite concentrations and mild, acute uterine infections, it is possible that prostaglandin $F_{2\alpha}$ metabolite concentrations may be used to help detect subclinical uterine infections in cattle. Detection of subclinical uterine infections may allow one to treat cows early and avoid the development of an infection that may impair reproductive performance.

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# SPONTANEOUS UTERINE INFECTIONS ARE ASSOCIATED WITH ELEVATED PROSTAGLANDIN $F_2\alpha$ METABOLITE CONCENTRATIONS IN POSTPARTUM DAIRY COWS

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## ABSTRACT

Postpartum Holstein (n=21) and Jersey (n=4) cows were used to determine if uterine infections are associated with elevated plasma concentrations of 13,14-dihydro-15-keto-prostaglandin $F_2\alpha$ (PGFM). Based upon clinical examinations and bacterial content of intrauterine fluid samples, cows detected with uterine infections between 21 and 28 d post partum were used (infected; n=14). These cows were matched with herdmates that were free of infection (control; n=11). Beginning on the day the cows were assigned to the experiment (Day 1), blood samples were collected on alternate days for the next 14 to 15 d. Plasma samples were stored at -20° C until assayed. From Day 1 until the end of the experiment, uterine fluid samples were collected transcervically twice weekly for aerobic bacterial culture. Endometrial biopsies were collected between Days 6 and 8 and Days 13 and 15. Control cows did not show signs of uterine infection throughout the trial, and bacterial cultures indicated that there were no significant bacterial populations in the uteri of the control cows. The uteri of infected cows harbored numerous microbes. Actinomyces pyogenes was most prominent. Various species of Streptococcus and Pasteurella were also prevalent in the infected cows. Escherichia coli was present in the uterus of both infected and control cows. Biopsies showed that infected cows had more ($P<0.05$) neutrophils, plasma cells and lymphocytes in the endometrium than did the control cows. As determined by plasma progesterone concentrations, 83% of the control and 50% of the infected cows had functional luteal tissue during the 2-wk sampling period. Plasma PGFM profiles were linear ($P<0.03$) and did not differ between treatment groups ($P>0.10$). However, average plasma PGFM concentrations were greater ($P<0.0001$) in infected than in control cows. These data indicate that plasma PGFM concentrations are greater in postpartum cows with spontaneous uterine infections then in herdmates free of infection.

Key words: endometritis, postpartum cows, prostaglandin $F_2\alpha$ metabolite

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## INTRODUCTION

Uterine prostaglandin $F_2\alpha$ release (as inferred from plasma 13,14-dihydro-15 keto prostaglandin $F_2\alpha$ concentrations; PGFM) is generally high during and immediately after parturition, and it gradually declines to basal concentrations by Day 20 post partum (5, 18, 19). Changes in postpartum plasma PGFM concentrations are correlated with the rate of uterine and cervical involution (13, 19, 20). Cyclic ovarian function also usually does not resume until concentrations of PGFM are basal (8, 19, 21). In addition, some reports indicate that there may be a relationship between endometritis and blood plasma concentrations of PGFM; however, data used to suggest this relationship were collected on only a small number of cows with spontaneous endometritis (20, 23, 35).

The bovine uterus usually harbors pathogenic bacteria during the first 2 wk after parturition (11). In most instances, by 3 to 4 wk after calving the uterus no longer contains pathogenic microbes. However, endometritis still accounts for approximately 20% of the reproductive disorders in postpartum cattle (4), and some reports have been as high as 67% (28). The principal bacterium consistently associated with uterine lesions and impaired reproductive performance is Actinomyces pyogenes (2, 11, 16, 22). Endometritis has a number of effects on reproductive performance. It delays uterine and cervical involution, and it lengthens the intervals from calving to ovulation, detected estrus, insemination, conception and return to basal PGFM concentrations (4, 10, 20). Therefore, the primary objective of this study was to determine if spontaneous uterine infections were associated with elevated blood plasma PGFM concentrations in postpartum cows.

## MATERIALS AND METHODS

A total of 148 Holstein (n=103) and Jersey (n=45) cows from the Virginia Polytechnic Institute and State University Dairy herd that calved between April 30, 1990, and February 13, 1991, were examined on Days 21 to 28 post partum by a single clinician. Palpation per rectum was performed to assess 1) the size and consistency of the cervix and uterus, 2) the presence of intrauterine fluid and 3) ovarian structures. The diameter (in millimeters) of the cervix and uterine horns, measured immediately cranial to the uterine bifurcation, was estimated by palpation. Visual inspection of the vestibule, vagina and external cervical os was performed through a sterile, disposable vaginal speculum to detect any purulent discharge. Cows with palpable intrauterine fluid and/or abnormal discharge were diagnosed as having a uterine infection. Cows with a normal tract upon palpation and a normal speculum exam were diagnosed as free of infection. Cows diagnosed with a uterine infection (infected; n=14) immediately placed on experiment (Day 1) and were matched with herdmates of the same breed and day (±1 d) post partum diagnosed as free of infection (control; n=11). Uterine fluid samples were collected transcervically twice weekly from all infected and control cows using a gas-sterilized (ethylene oxide) infusion pipette covered with a protective outer sheath to prevent vulval and vaginal contamination. A syringe was attached to the end of the pipette to aspirate uterine fluid. Each sample was transferred immediately into a sterile glass vial, capped and transported to the clinical microbiology laboratory for aerobic bacterial culture on Columbia Blood Agar plates. Subsequent identification of microorganisms was conducted using standard

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routine procedures based on morphology and biochemical characteristics (3) in order to verify the presence of pathogenic bacteria. The bacteriological data was used in support of the clinical examination.

Two endometrial biopsies were collected transcervically; the first was collected between Days 6 and 8, the second between Days 13 and 15 after cows were assigned to the experiment. Biopsies were obtained from the dorsomedial aspect of the right horn just cranial to the bifurcation using a sterile alligator-jawed uterine biopsy forceps with a 4 x 12-mm basket. Biopsy specimens were placed immediately into Karnovsky's fixative (approximately 3 ml). After a 24-h fixation period, endometrial biopsies were transferred to a standard phosphate buffer solution (approximately 5 ml) and stored until prepared for histological evaluation as described by Griffin et al. (11).

Beginning on the day of assignment to the experiment (Day 1) and continuing for the next 14 to 15 d, blood samples were collected from the medial caudal vein in the tail on alternate days. Within 45 min after collection, the samples were centrifuged at 1000 x g for 25 min. Plasma was collected and stored at -20°C until assayed for PGFM using enzymeimmunoassay (EIA) procedures described by Del Vecchio et al. (6) and for progesterone ($P_4$) using RIA procedures described by Harrison et al. (15). The inter- and intra-assay coefficients of variation for PGFM and $P_4$ were 13.1, 5.1, 8.2 and 5.5%, respectively.

If deemed necessary at the end of the experiment, infected cows were treated with prostaglandin $F_2\alpha$ (25 mg i.m., Lutalyse, The Upjohn Co., Kalamazoo, MI) to aid resolution of the endometritis. All cows were either returned to the herd for rebreeding or were culled based upon previous performance records. Most of the cows in this study were not returned to the breeding herd, so subsequent reproductive performance records could not be attained.

Data were analyzed with the General Linear Models procedures in SAS (29). Split-plot analyses of variance with day as a continuous independent variable and tests for homogeneity of residual variances were used to determine the effect of endometritis on changes in PGFM concentrations over time during the experiment. Polynomial regression analysis was used to determine the shape of PGFM curves. Proportional data for the number of cows with functional luteal tissue and frequency of bacterial isolates were analyzed with Fisher's Exact Test (31). Numbers of neutrophils, plasma cells, lymphocytes and macrophages present and the degree of fibrosis in the endometrium were analyzed with the Mann-Whitney Test (30). Data are reported as least-squares means ± SEM or as percentages.

## RESULTS

Uterine fluid samples from the infected cows contained numerous microbes. Actinomyces pyogenes was the most prominent. Various species of Streptococci and Pasteurella were also prevalent in the uteri of the infected cows (Table 1). Escherichia coli was present in the uteri of both the infected and control cows. Control cows did not show clinical signs of endometritis, and the uterine fluid samples from these cows did not contain any significant bacterial pathogen throughout the trial.

Endometrial biopsies indicated that the infected cows had greater ($P<0.05$) numbers of neutrophils, plasma cells and lymphocytes in the endometrium than did the control cows. The number of macrophages and degree of fibrosis in the endometrium were similar ($P>0.10$) in the infected and control cows.

Table 1. Summary of the type and frequency of appearance of bacteria isolated from the uteri of infected and control cows. Percentile data represent the frequency of appearance of specific bacteria from samples collected twice weekly during the 14 to 15 d sampling period.

| Type of bacteria | Frequency of appearance (%) | |
|---|---|---|
| | Infected cows | Control cows |
| Actinomyces pyogenes | 64.3 (9 of 14)[a] | 0.0 (0 of 11)[b] |
| Streptococcus | 57.1 (8 of 14)[a] | 18.2 (2 of 11)[b] |
| S. viridaus | (2 of 14) | |
| S. agalactiae | (1 of 14) | |
| S. bovis | (1 of 14) | (1 of 11) |
| S. group D | (3 of 14) | (1 of 11) |
| S. dysgalactiae | (1 of 14) | |
| Escherichia coli | 35.7 (5 of 14) | 63.6 (7 of 11)* |
| Pasteurella | 28.6 (4 of 14) | 9.1 (1 of 11) |
| P. spp | (2 of 14) | (1 of 11) |
| P. hemolytica | (1 of 14) | |
| P. pneumotropica | (1 of 14) | |
| Staphylococcus | 14.3 (2 of 14) | 9.1 (1 of 11)* |
| S. intermedius | (1 of 14) | |
| S. coagulase | (1 of 14) | (1 of 11) |
| Bacillus | 7.1 (1 of 14) | 18.2 (2 of 11)* |
| B. spp | | |
| Coryneforms | 7.1 (1 of 14) | 18.2 (2 of 11)* |

* Only a few colonies were present and bacterial growth was insignificant (less than 1+).
[a,b] Different superscripts within rows indicate a difference at $P<0.05$.

The occurrence of progesterone concentrations above 1 ng/ml indicated that 83% of the control and 50% of the infected cows had functional luteal tissue during the 2-week sampling period; the percentages were not different. Average diameter of the right and left uterine horns was greater ($P<0.06$) in infected than in control cows, but the diameter of the cervix was similar in the two groups of cows (Table 2).

Table 2. Average (mean ± SEM) diameter (mm) of the right and left uterine horns and cervix of control and uterine infected cows at the time of assignment to the experiment (i.e., Days 21 to 28 post partum)

| Item | Infected | Control |
|---|---|---|
| Right uterine horn | 32.5 ± 2.7[a] | 24.9 ± 1.5[b] |
| Left uterine horn | 31.0 ± 3.3[a] | 23.7 ± .8[b] |
| Cervix | 34.2 ± 4.3 | 27.2 ± 1.2 |

[a,b]Different superscripts within rows indicate a difference at $P<0.06$.

Prostaglandin $F_2\alpha$ metabolite profiles were linear and did not differ between the infected and control cows. However, average overall concentrations of PGFM were greater ($P<0.0001$) in infected (261.5 ± 21.7 pg/ml) than in control (205.4 ± 14.9 pg/ml) cows (Figure 1). As shown in Figure 1, the difference in PGFM concentrations appear to be greatest early, with a decline between Days 3 and 5. This decline could be due to the withdrawal of purulent exudate when collecting the uterine fluid samples, which may have reduced the infectious insult on the uterus.

## DISCUSSION

The results of this study indicate that postpartum cows with spontaneous uterine infections have higher blood plasma concentrations of PGFM than do contemporaneous herdmates without uterine infections. Results of the present study agree with some of the earlier findings indicating that postpartum cows with endometritis have higher basal PGFM concentrations than their herdmates without an infection (35). Peter and Bosu (23) have also reported that increases in PGFM concentrations following first postpartum ovulation were associated with moderate to heavy intrauterine bacterial growth patterns and infection. In contrast, Vighio et al. (34) reported that cows with pyometra had PGFM concentrations that were similar to noninfected controls.

Additional data on the relationship between uterine infections and PGFM have been reported utilizing postpartum cows with experimentally induced uterine infections (5) or given intrauterine infusions of E. coli endotoxin (24). In the study by Del Vecchio et al. (5), increases in jugular plasma PGFM concentrations and changes in the PGFM profiles were observed over



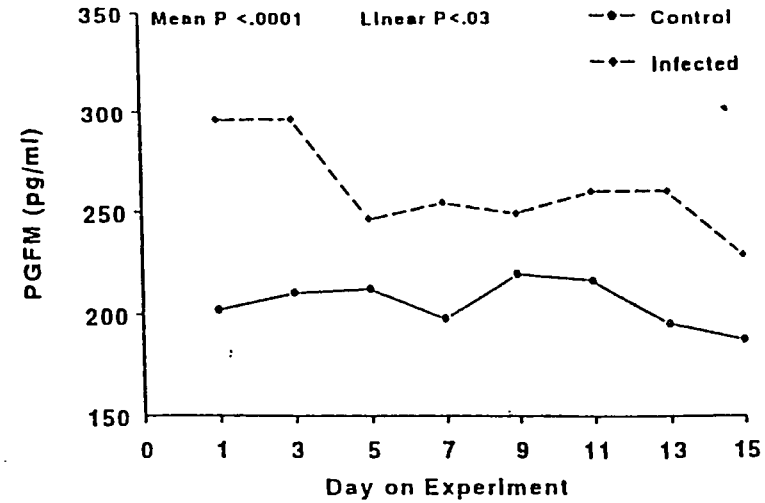


Figure 1. Average plasma 13,14-dihydro-15-keto-prostaglandin $F_2\alpha$ (PGFM) concentrations in control and infected cows from Day 1 until the end of the experiment. Mean concentrations of PGFM were greater ($P<0.0001$) in infected than in control cows. Shape of the PGFM curves were linear and similar ($P>0.10$) between the 2 groups of cows. The least-squares means SEM was 14.9 pg/ml for both groups.

approximately a 2 wk period in association with intrauterine bacterial infusions and subsequent endometritis. Peter et al. (24) reported a short-lived (4 h) increase in plasma PGFM when cows were treated on Day 5, but no increase in PGFM was observed when cows were treated on Day 20 post partum. With the present study specifically designed to test whether PGFM concentrations are higher in postpartum cows with uterine infections, and based on results from the previous work mentioned, we feel these data accurately depict the relationship between plasma PGFM and uterine infections in postpartum cattle.

The physiological relevance of increased PGFM concentrations in response to uterine infections is unclear. Some researchers (23) have hypothesized that the increases in uterine prostaglandin production may cause the premature demise of an existing corpus luteum which will result in lowered progesterone concentrations. Progesterone has a quiescent effect on the uterus (27) and has been shown to posses direct and indirect immunosuppressive properties (12, 14). Therefore, by secreting prostaglandin $F_2\alpha$ ($PGF_2\alpha$) the uterus may be acting to terminate corpus luteum function and lower progesterone concentrations in an attempt to rid itself of the infectious organisms. However, some researchers reported a prolonged luteal phase associated with postpartum uterine infections, but this occurs when the uterine infection develops into pyometra (9, 10, 34). In the case where there is no corpus luteum present and progesterone is

low, $PGF_2\alpha$ may stimulate the myometrium to contract and physically force the infection out of the uterus through strong peristaltic movements (17, 26).

Although $PGF_2\alpha$ is generally regarded as a luteolysin in cattle, some studies have shown many beneficial effects of $PGF_2\alpha$ in postpartum cows without a corpus luteum present on the ovary or elevated progesterone (1, 7, 25, 33). It is possible that $PGF_2\alpha$ of uterine origin has numerous modes of action that are not yet understood which hasten completion of the postpartum anestrous period.

In the present study, the most common bacteria found in the uterus of the infected cows was A. pyogenes, which concurs with previous studies (11, 32). Actinomyces pyogenes has been consistently reported to have a close association with abnormalities of the endometrium (2, 11). The data from this study indicate anomalies in the endometrium of the infected as compared to the control cows. More specifically, there appeared to be an association between cows harboring A. Pyogenes and higher degrees of lymphocyte, plasma cell and neutrophil infiltration within cows in the infected group.

Given the apparent relationship between blood plasma PGFM and uterine infections, it is possible that PGFM may be used to help detect subclinical uterine infections in cattle. With modifications to a recently developed enzymeimmunoassay for PGFM (6) it may be possible to detect PGFM in milk as well as in plasma. Respective samples can be collected during scheduled milking periods and assayed for PGFM within 12 to 24 h. If an animal is found to have elevated PGFM concentrations then this could be perceived as indicative of a uterine problem. Clearly more work needs to be conducted in order to test the feasibility of using PGFM as an indicator of uterine infections. Detection of subclinical uterine infections may allow for early treatment of cows developing uterine infections and avoid the development of an infection that may impair reproductive performance.

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# JUGULAR PLASMA CONCENTRATIONS OF 13,14-DIHYDRO-15-KETO-PROSTAGLANDIN $F_{2\alpha}$ IN PREPUBERTAL BEEF HEIFERS TREATED WITH PROGESTOGEN THEN CHALLENGED WITH OXYTOCIN

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## ABSTRACT

Prepubertal Angus crossbred heifers (n=24) between 8 and 10 mo of age were used to determine if progestogen treatment would enhance jugular concentrations of 13,14-dihydro-15-keto-prostaglandin $F_2\alpha$ (PGFM) after oxytocin (OT) injections. Heifers were stratified by age and weight and allotted to randomized treatments in a 2 × 2 factorial arrangement. Heifers were treated with either a norgestomet (NOR) implant (6 mg) for 9 d or no implant (0 mg; BLK). On d 8 of NOR treatment, jugular veins were catheterized and, on d 9, blood samples were collected every 15 min for 165 min. The first four samples were used to determine basal PGFM concentrations (an indirect measure of uterine $PGF_2\alpha$ release). After collection of the fourth sample, either OT (100 IU) or saline (0 IU; SAL) was injected via the jugular catheter. After the 165-min sample was collected, NOR implants were removed. Beginning 48 h after implant removal, a second 165- min blood sampling period was initiated. Average progesterone concentrations were less than 1 ng/ml during both bleeding periods. Within treatment, PGFM concentrations were similar between the first and second sampling periods; therefore, data within treatment were combined. Basal PGFM concentrations were higher ($P<.01$) in NOR-treated than in BLK heifers. Oxytocin did not increase PGFM concentrations in BLK-OT heifers; however, a marked increase in PGFM was detected in the NOR-OT heifers in response to oxytocin. Average PGFM concentration was greatest ($P<.0001$) in NOR-OT heifers, and PGFM profiles differed ($P<.0001$) between NOR-OT and each of the other treatment groups. Results from this study indicate that NOR increases basal PGFM and may "condition" the uterus to respond to OT in prepubertal heifers.

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## INTRODUCTION

Research on the endocrine events associated with puberty has focused primarily on the hypothalamic - pituitary - ovarian axis (1 - 8). Few studies have been conducted to determine the physiological and endocrinological relationships between the uterus and ovaries around the time of puberty in heifers (9).

Studies with ovariectomized heifers and ewes (10 - 13) and intact cyclic heifers (14, 15) indicated that estrogen, progesterone and oxytocin are regulators of uterine prostaglandin secretion. A major function of the uterus during the estrous cycle is to regulate luteal lifespan; prostaglandin $F_2\alpha$ ($PGF_2\alpha$) is believed to be the endogenous uterine factor that causes cyclic luteolysis (16). Endometrial $PGF_2\alpha$ and luteal oxytocin appear to be part of the uterine-ovarian mechanism that causes luteolysis in cattle (17).

Although transient increases in circulating progesterone concentrations have been detected 1 and 3 wk before the first estrus in heifers (18, 19), the physiological significance of these changes has not been determined. Oxytocin injections just before puberty increase jugular concentrations of 13,14-dihydro-15-keto-prostaglandin $F_2\alpha$ (PGFM) in heifers; however, a PGFM rise is not detected in all heifers, and it is less than increases reported for cows (20). Perhaps prepubertal increases in progesterone initiate uterine endocrine function, i.e., $PGF_2\alpha$ synthesis and release.

The objectives of this study were to 1) mimic experimentally a prepubertal rise in progesterone and, 2) determine whether the progestogen treatment enhances the ability of oxytocin to increase jugular PGFM concentrations in prepubertal heifers.

## MATERIALS AND METHODS

Prepubertal Angus crossbred heifers (n=24) were maintained as a group and fed a balanced diet sufficient to support daily weight gains of at least .75 kg/hd. Beginning when age averaged 225 d, heifers were checked for signs of estrus from approximately 0700 to 0730 and 1600 to 1630 daily. Heifers were considered prepubertal if they were not detected in estrus during the 6- to 8- wk period before treatments were first administered. Heifers were stratified by age and weight and allotted to randomized treatments in a 2 x 2 factorial arrangement (Table 1). The main effects were progestogen (0 vs. 6 mg norgestomet; 17α-acetoxy-11ß-methyl-19-nor-preg-4-ene-3,20-dione; Sanofi AgriVet, Overland Park, KS) and oxytocin (0, saline vs 100 IU; Ampro Pharmaceutical, Arcadia, CA). Norgestomet was implanted subcutaneously in the right ear and left in place for 9 d. Heifers were halter broken and conditioned to stand tied in a barn. On d 8 of norgestomet treatment, the heifers were fitted with indwelling jugular catheters and, on d 9, blood samples were collected at 15-min intervals for 165 min. Heifer were kept tied while



Table 1. Average age and weight (mean ± SE) of heifers used in the experiment.

| Treatment | Number[a] | Age (d) | Weight (kg) |
|---|---|---|---|
| Norgestomet and oxytocin | 6 | 288.7 ± 1.2 | 287.0 ± 7.3 |
| Norgestomet and saline | 6 | 286.3 ± 1.9 | 278.6 ± 4.6 |
| No implant/blank and oxytocin | 6 | 285.3 ± 2.0 | 286.1 ± 6.5 |
| No implant/blank and saline | 6 | 283.3 ± 3.2 | 273.0 ± 5.8 |

[a]Number of heifers in each treatment group.

blood samples were being collected. The first four blood samples were used to determine basal PGFM concentrations. After the fourth sample was collected, either oxytocin or saline was injected into the heifers via the jugular catheter. At the end of the sampling period, norgestomet implants were removed. The jugular catheters were secured, and the heifers were released into a small pasture. A second 165-min blood sampling period was initiated, including i.v. oxytocin or saline, 48 h after implant removal. Blood samples were collected with syringes, transferred immediately into test tubes containing 100 USP units of heparin (Sigma Chemical Co., St. Louis, MO) and chilled to 4°C. Within 45 min after collection, all samples were centrifuged at 800 x g for 20 min. Plasma was collected and stored at -20°C until assayed for PGFM (enzymeimmunoassay; 21) and progesterone (radioimmunoassay; 22). Inter- and intra-assay coefficients of variation were 10.8% and 5.5% for PGFM and 4.0% and 3.3% for progesterone, respectively. Jugular PGFM concentrations were used as an indirect measure of uterine $PGF_2\alpha$ secretion.

Data were analyzed with the General Linear Models procedures of SAS (23). Polynomial regression analysis was used to determine the shape of the PGFM curves (concentration vs. time). Analyses of variance with time as a continuous independent variable and tests for homogeneity of residual variances were used to determine the effect of treatment on changes in PGFM concentrations over time (24). Separate analyses of variance were used to compare basal PGFM and progesterone concentrations between treatments and bleeding periods. The least-squares means (LSM) predicted difference option in SAS, which tests the Ho: $LSM_i = LSM_j$, was used to separate means. Within treatment PGFM and progesterone concentrations were similar between the first and second blood sampling periods; therefore, within treatment PGFM and progesterone data from the two sampling periods were combined.

## RESULTS

Treatment did not affect the average concentrations of plasma progesterone; they were less than 1 ng/ml in all treatment groups throughout the study (Table 2). However, two heifers in the BLK-OT group and one heifer in the NOR-OT had progesterone concentrations greater than 1 ng/ml during both sampling periods. Because the PGFM profiles for those heifers were similar to profiles for other heifers in their groups, PGFM data for all heifers were used were used to evaluate the effects of treatment.

Norgestomet increased ($P < .01$; main effect of norgestomet vs. blank) the basal (i.e., average of the first four samples before oxytocin or saline) concentration of PGFM (norgestomet, 269.0 ± 8.6 pg/ml; blank, 238.1 ± 8.6 pg/ml). For the overall mean concentrations of PGFM, there was a norgestomet × oxytocin interaction ($P < .0001$; Table 2). The overall average concentration of PGFM in NOR-OT-treated heifers was greater than that in heifers in the other treatment groups (Table 2).

Table 2. Plasma concentrations (overall mean ± SE) of progesterone (ng/ml) and 13,14-dihydro-15-keto-prostaglandin $F_2\alpha$ (PGFM; pg/ml) in prepubertal heifers.

| Treatment[a] | PGFM[b] | Progesterone |
|---|---|---|
| Norgestomet and oxytocin | 468.2 ± 10.5[c] | .64 ± .52 |
| Norgestomet and saline | 270.0 ± 10.5[d] | .19 ± .06 |
| No implant/blank and oxytocin | 231.2 ± 10.5[e] | .52 ± .37 |
| No implant/blank and saline | 228.0 ± 10.5[e] | .16 ± .05 |

[a]Sampling period did not affect PGFM or progesterone concentrations; therefore, data for the first and second sampling periods were combined within treatment. Three heifers (2 blank and oxytocin; 1 norgestomet and oxytocin) had progesterone values greater than 1 ng/ml.
[b]There was a norgestomet × oxytocin interaction ($P < 0001$).
[c,d,e]PGFM means with different superscripts differ ($P < .01$).

For profiles of PGFM concentrations, there was a norgestomet × oxytocin interaction ($P < .0001$; Figure 1). In NOR-OT treated heifers, PGFM increased from approximately 275 pg/ml just before i.v. injection of oxytocin to greater than 450 pg/ml within 15 min after oxytocin (Figure 1). Concentrations of PGFM increased steadily and peaked at about 800 pg/ml at 75 min after oxytocin, then declined during the remainder of the sampling period. Norgestomet alone affected ($P < .01$) the shape of the response curve. In NOR-SAL treated heifers, PGFM increased slightly



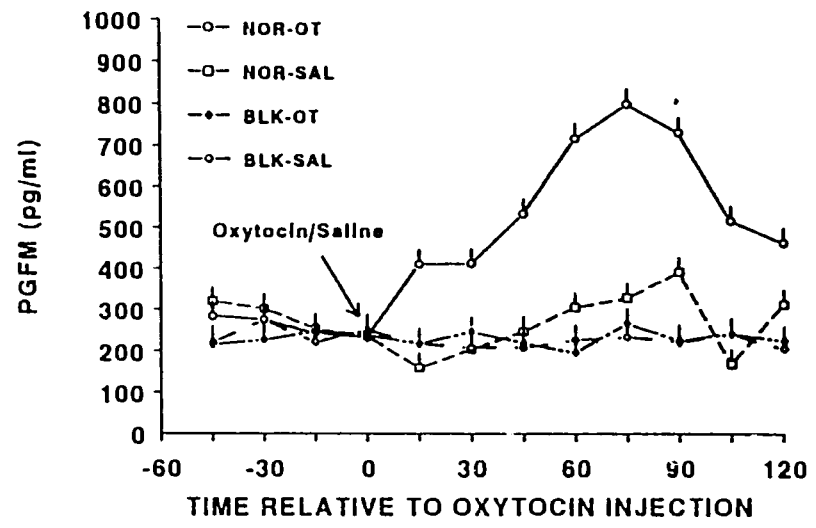


Figure 1. Plasma PGFM concentrations for heifers in response to oxytocin (OT) and norgestomet (NOR). The arrow indicates the time of OT or saline (SAL) treatment. Blood samples were collected at 15-min intervals. Oxytocin alone did not affect the PGFM profile in heifers not treated (BLK) with norgestomet (BLK-OT vs. BLK-SAL). The PGFM profile of NOR-SAL-treated heifers differed ($P < .01$) from that for BLK-OT and BLK-SAL-treated heifers. The PGFM profile differed ($P < .0001$) between NOR-OT-treated heifers and the profiles for all other treatment groups.

during the sampling period; the greatest concentration was about 400 pg/ml (Figure 1). In heifers not treated with norgestomet (i. e., BLK-OT vs. BLK-SAL) the concentrations of PGFM remained basal during the sampling period (Figure 1).

## DISCUSSION

Oxytocin treatment alone did not affect jugular plasma PGFM concentrations; however, after 9 d of norgestomet treatment, oxytocin induced a four-fold increase in peak concentrations of PGFM. These data differ slightly from an earlier study in which 45% of the prepubertal dairy heifers, of similar age to those in the present study but without progestogen, responded to an oxytocin challenge (20). Progesterone data were not available, so whether the dairy heifers that responded to oxytocin had been exposed to transient increases in endogenous progesterone is not known.

Typically, dairy (Holstein) heifers attain puberty at an earlier age (304 d; 9) than beef (Angus) heifers (367 to 394 d; 25). Thus, prepubertal increases in progesterone would occur at an earlier age, possibly influencing the ability of dairy heifers to respond to oxytocin.

Previous studies indicate that jugular PGFM may be used as a general indicator of uterine $PGF_2\alpha$ release (26, 27) and progesterone is a potent, chronic regulator of uterine $PGF_2\alpha$ secretion (for review, see Silvia et al. 28). Carver (29) measured $PGF_2\alpha$ release in intact and progesterone-treated ovariectomized cows and reported no differences in $PGF_2\alpha$ secretion between the two groups. Others (30, 31) reported similar data for ewes. Progestogen treatment elevates uterine $PGF_2\alpha$ production in anestrous postpartum beef cows (32) and, when given during estrus or metestrus, increases in PGFM were detected earlier in the estrous cycle; PGFM increases were associated with a premature decline in plasma progesterone and a shortened estrous cycle (33).

Oxytocin stimulates endometrial $PGF_2\alpha$ secretion in intact and ovariectomized cows after the uterus has been under a progestational influence for at least 7 d (13, 15); this effect occurs in the absence of estradiol supplementation. Progestogen treatment, for periods of 7 d or longer, may increase oxytocin receptor concentrations. Vallet et al. (34) found that the number of uterine oxytocin receptors is increased in ovariectomized ewes treated with progesterone for 12 d. In cyclic cows, endometrial oxytocin receptor numbers are negatively correlated with progesterone concentrations, but the PGFM response to oxytocin was not correlated with oxytocin receptor numbers (35, 36). Those data indicate that endometrial secretion of $PGF_2\alpha$ is not necessarily limited by the number of oxytocin receptors. Although endometrial oxytocin receptor concentrations were not measured in the present study, jugular plasma PGFM increases in response to the oxytocin challenge reflect an increase in endometrial $PGF_2\alpha$ secretion. The 9 d norgestomet treatment may have influenced endometrial oxytocin receptor concentrations or some other mechanism (e.g., initiated uterine contractions; 37) which stimulated endometrial $PGF_2\alpha$ release in response to oxytocin.

In prepubertal heifers, there may be a signal or stimulus that conditions the uterus to recognize and respond to oxytocin before the initiation of cyclicity. Norgestomet treatment, without oxytocin, increased plasma PGFM concentrations which indicates that a period of progestational conditioning affects uterine endocrine function in prepubertal heifers. Results from the present study indicate that mimicking a prepubertal rise in progesterone, increases basal PGFM and enhances the effectiveness of oxytocin to increase plasma PGFM concentrations. Based upon the results of this study, we believe that the transient increases in progesterone measured 1 and 3 weeks before puberty (18, 19) may prepare the uterus to perform an endocrine role (i. e., enhanced $PGF_2\alpha$ synthesis and release) necessary for cyclic ovarian function.



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# Uterine Health and Disorders: Uterine Infections

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## ABSTRACT

Nonspecific uterine infections reduce reproductive efficiency and profit potential of dairy cows. Fortunately, most cows prevent severe uterine infections. The term, uterine infection, implies that the uterus is contaminated with pathogenic organisms. Indeed, *Actinomyces pyogenes*, either alone or with other bacteria, is often associated with uterine infections. When *A. pyogenes* was isolated from uterine fluids after d 21 postpartum, cows developed severe endometritis and were infertile at first service. Nevertheless, exact causes of uterine infections are not known, but they are associated consistently with several factors. Cows with dystocia, retained placentas, twins or stillbirths, and various metabolic disorders are more likely to develop metritis than are other cows. Aberrant immune function before and after calving seems to predispose cows to severe uterine infections. Few cows die from uterine infections, but they are more likely to be culled for poor reproductive performance. Uterine infections can reduce milk yield, and some treatments contaminate milk and force it to be discarded. Because they are nonspecific, uterine infections are difficult to prevent; attention to sanitation and periparturient hygiene, especially during assisted calving, may be the best defense. Evidence that aberrant immune function predisposes cows to uterine infections indicates that methods for regulating immune function in periparturient cows has potential for preventing and(or) treating uterine infections.



**(Key words:** cow, uterine infection, endometritis, metritis, pyometra, immune function)

# INTRODUCTION

Uterine disorders, primarily nonspecific uterine infections, reduce the reproductive efficiency of dairy cows. In some herds, 40% of the postpartum cows may be diagnosed with, and treated for, uterine infections. In addition to reducing reproductive efficiency, uterine infections usually increase herd health costs, and they often reduce feed consumption, cause an appreciable reduction in milk yield, and force producers to cull cows that would otherwise be productive and remain in the herd. A 1986 report indicates that each lactating dairy cow with a uterine infection (i.e., metritis) cost a producer $106 (3). Clearly, uterine infections can have a major impact on the profitability of a dairy operation.

The purpose of this review is to describe 1) uterine infections and diagnostic tools, 2) factors that predispose cows to uterine infections, 3) some treatment strategies, but not describe or recommend specific treatments, 4) some of the consequences of uterine infections, and 5) some of the possible endogenous mechanisms associated with uterine infections. The review is intended to be an overview rather than a detailed evaluation of each reference.

## Definitions and Diagnostic Tools

The scientific literature contains, literally, thousands of publications with some reference to uterine infection; most of those publications are more relevant to human beings than to livestock, and more of the references are relevant to mares than to food-producing animals. In addition, uterine infection is a rather general term, and the criteria used to diagnose and classify uterine infections seem to vary among investigators. In fact,

many authors do not describe their criteria for diagnosing and classifying uterine infections, so comparisons among studies and interpretation of data are often difficult. Thus, for the purpose of this review, uterine infections will be classified, when possible, according to period postpartum and clinical signs, but, when appropriate and for brevity, the term also will be used to encompass all types of nonspecific uterine infections.

Olson et al. (48) defined the postpartum period as the interval from parturition to complete uterine involution, and they divided the postpartum period into three subperiods: puerperal period, intermediate period, and postovulatory period. Puerperal period was defined as the interval from calving until the pituitary becomes responsive to GnRH (i.e., at approximately 7 to 14 d postpartum). Intermediate period is the interval from when the pituitary becomes responsive to GnRH to first postpartum ovulation. Postovulatory period is the interval from first ovulation to complete uterine involution, which was defined loosely as when the uterine horns and cervix were approximately 40 mm in diameter and the caruncles were epithelialized; involuted uterine horn and cervical diameters of approximately 40 mm are consistent with several studies (e.g., 35). Even though the definition of each postpartum subperiod is somewhat less precise than some may prefer, the definitions are functional and consistent with classifications of uterine infections.

Uterine infections are generally classified according to clinical signs and degree of severity (69). The term, uterine infection, implies that the uterus is contaminated with pathogenic organisms. Endometritis indicates that the endometrium is inflamed, and, when it occurs after the puerperal period, endometritis is considered the least severe

classification of uterine infection. Metritis indicates that all layers of the uterine wall are inflamed, and pyometra indicates that purulent exudate has accumulated in the uterine lumen. Endometritis, metritis, and pyometra are related (i.e., they may develop sequentially) and often called collectively metritis-pyometra complex, or metritis complex (3, 48).

During the puerperal period, perhaps 90% of the cows develop a mild nonpathological endometritis (2, 28, 48), which seems to be a characteristic of uterine involution (50). Typically, all palpable uterine fluids (lochia) are voided during the first 2 wk postpartum (48). Lochia varies in color from whitish to reddish to dark brown, and the color of lochia is not a reliable sign of a problem. However, if the lochia becomes fetid, puerperal metritis may be suspected. Puerperal metritis can be a severe problem, and life-threatening uterine infections (i.e., septic-toxic metritis) are associated almost exclusively with this condition. Often, puerperal metritis requires systemic treatments, because endotoxins and pathogenic organisms can escape from the uterus. Cows with septic-toxic metritis usually recover or die within 2 to 10 d (43).

During the intermediate period, "normal" cows reduce or eliminate pathogenic organisms that are found typically in the postpartum uterus (48). Uterine infections that persist during this period are often classified as endometritis or metritis, and both conditions can become chronic (2). When cows with chronic endometritis or metritis ovulate (i.e., beginning of the postovulatory period), pyometra often develops within a few days (2). Indeed, pyometra is detected almost exclusively in cows with active corpora lutea, and some cows develop pyometra after uterine involution is complete (50, 69).

Pyometra seems to prevent the uterus from releasing luteolytic amounts of $PGF_{2\alpha}$, so the condition typically induces persistent corpora lutea. In turn, persistent corpora lutea produce a uterine environment that permits pyometra to persist.

Timely and accurate diagnosis are essential for assuring appropriate management of uterine infections. However, some authors suggest that diagnoses of uterine infections are too subjective and often inaccurate (25, 50). Rectal palpation of the uterus, examination of the vagina with a speculum, culture of uterine fluids, and uterine biopsies are the techniques used to diagnose uterine infections (6). Rectal palpation is probably the most common method for diagnosing uterine infections, but it may be the most insensitive nonspecific error-prone method used (6, 25). Typically, one attempts to judge the size and consistency of the uterus and cervix and fluid content of the uterus relative to the characteristics considered "normal" for the given time postpartum (6, 69). Undoubtedly, the ability to use rectal palpation of the uterus to accurately diagnose infections is closely related to skill and training, which vary tremendously among practitioners. Thus, one should expect rectal palpation to be an error-prone diagnostic tool.

Examination of the vagina with a speculum is a straightforward procedure that allows one to evaluate the characteristics of fluids in the anterior vagina and external cervical os (6). Vaginal discharge can be scored and used as an indicator of uterine infection (32). However, even though diagnoses with this procedure are more consistent with diagnoses based on bacterial isolation than are those based on rectal palpation alone, vaginal examination seems to be used less frequently than rectal palpation (6, 25). Vaginoscopic examination combined with palpation of the uterus should increase the



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accuracy of diagnosing uterine infections, but, as described clearly (25, 26), well-designed and controlled studies to evaluate diagnostic procedures suitable for field use are lacking.

Uterine fluids can be swabbed and cultured to characterize the bacterial populations during the postpartum period. Anaerobic and aerobic bacteria are associated with uterine infections (48), so sampling procedures should be consistent with the two types of organisms. Even though cultures of uterine fluids are informative, they do not provide definitive evidence that a cow should be treated for uterine infection (6). Also, cultures of uterine fluids are more costly and less timely than most producers would prefer.

Uterine (i.e., endometrial) biopsies are informative. In fact, cultures from biopsies and cultures from swabs gave similar information about the uterine microflora of postpartum cows (46). But, the uterine biopsy procedure has many of the same diagnostic limitations as cultures of uterine fluids (6). In addition, there are other limitations of uterine biopsies (6, 25): to be most informative, several sites should be biopsied; biopsies should be collected on more than one day postpartum; biopsies and harsh uterine manipulation can impair fertility. In spite of the diagnostic limitations of uterine biopsies, they may provide meaningful prognostic information about the reproductive potential of cows with uterine infections, but the practicality of that is questionable (27, 28).

To summarize, the general classifications of uterine infections (i.e., endometritis, metritis, and pyometra) have been somewhat standardized, the terminology seems to be used universally, and the general procedures used to diagnose uterine infections seem somewhat standardized. Although, the ability to use the diagnostic procedures, especially

rectal palpation of the uterus and vaginoscopic examination, varies considerably. Indeed, the typical diagnosis of a uterine infection seems rather imprecise. Because few authors describe how they determine whether a cow truly has a uterine infection, or how they classify an infection as endometritis, metritis, or pyometra, the interpretation of data is often difficult.

**Incidence**

The incidence of uterine infections varies considerably among studies, and the average incidence of uterine infections is not especially meaningful. That is not surprising, because many authors did not indicate how uterine infections were diagnosed, the classification of the uterine infections, the postpartum period during which the infections were detected, the parity of the cows, the general characteristics of the cows, or herd management practices. For example, of the cows examined between 5 and 14 d postpartum in seven herds, 36% were diagnosed with metritis, and the range across herds was 24.8 to 51.3%; apparently, there was an effect of herd (39). In contrast, of the cows examined once during the first 30 d postpartum in 31 herds, 7.8% were diagnosed with metritis, but the range in rates across herds was not reported and the metritis classification included endometritis, metritis, and pyometra (12).

Would the rates have been comparable if the cows had been examined on the same schedule, or if the data had been sorted according to type of cow, management practices, etc.? Based on cultures of uterine fluids and uterine biopsies, the incidence of endometritis, usually mild, seems to be greater during the first 14 d postpartum than during d 29 to 35 (28). However, the incidence of severe endometritis, which often

progresses to pyometra (2), increased at approximately the time (i.e., d 15 to 28 postpartum) when many cows would be expected to enter the postovulatory period (23), and, by d 43 to 49, the incidence of severe endometritis had decreased to 8% (28). Also, the incidence rate of metritis during consecutive 21-d intervals decreased as the interval from calving increased (19). The intent of this discussion is not to criticize specific studies; indeed, the two studies cited to illustrate the variation in the reported incidence of metritis provide important insights into factors that predispose cows to metritis. Instead, the intent is to indicate the need for more consistent definitions of uterine infections, examination periods postpartum, descriptions of cows and herd management practices, etc., so that one can put the results of various studies into proper perspective.

**Predisposing and Associative Factors**

The exact causes of postpartum uterine infections are not known. Pathogenic organisms isolated from infected uteri are found generally in livestock environments and are capable of infecting other tissues and organs. Thus, uterine infections are classified as nonspecific infections; in contrast, chlamydiosis is considered a specific infection. In spite of the nonspecific classification, a considerable amount of literature indicates that uterine infections are usually associated with certain factors. The word "associated" should be noted, because the specific causal factors, if they are actually specific, are not clear.

During the puerperal period, bacteria invade the uteri of at least 90% of the cows, and the composition of the uterine flora fluctuates constantly during the first 7 wk after calving (i.e., the entire postpartum period; 28). The uterus seems to repeatedly become contaminated, clear the organisms, and become recontaminated until uterine involution is

complete (28). The majority of the cows resolve endometritis spontaneously, and normally there is no appreciable effect on reproductive performance or any other measure of productivity. However, when a uterus becomes infected (i.e., unable to readily clear the organisms and the organisms proliferate), inflammatory cells infiltrate endometrium, superficial epithelium may desquamate and become necrotic, endometrium becomes hyperemic and congested, and number of plasma cells, neutrophils, and lymphocytes in endometrium increase (6, 14, 64). Uterine glands may become cystic, there may be periglandular fibrosis, uterine glands may atrophy, and scar tissue may replace functional endometrium (6, 14, 64). Inflammatory exudates can accumulate in the uterus, and infection spreads to the oviducts in 70 to 75% of the cows (6, 48). Oviducts are not as resilient as the uterus, and they easily sustain permanent damage (6). When endometritis progresses to pyometra, the uterus accumulates a considerable amount of purulent exudate, which is usually palpable because the cervix is typically constricted enough to prevent the exudate from draining freely (2). The extent of the changes in the uterus varies considerably among cows and with severity of infection. Often, especially with pyometra, there is no immediately noticeable sign that a cow has a uterine infection.

Because composition of uterine flora changes somewhat at each recontamination, no specific combination of organisms is associated consistently with postpartum uterine infections (28, 59). Nevertheless, *Actinomyces pyogenes*, either alone or in combination with other bacteria such as the anaerobes *Fusobacterium necrophorum* and *Bacteroides* ssp. (22, 59), is often associated with uterine infections (14, 28, 59). When *A. pyogenes* was isolated from uterine fluids after approximately d 21 postpartum, cows developed

severe endometritis and were usually infertile at first service; after d 22 postpartum, *A. pyogenes* was isolated from only one cow that conceived to first service (28). In addition, Griffin et al. (28) reported that 69% of the cows with uterine infections harbored *A. pyogenes*, often in pure cultures, and Del Vecchio et al. (14) reported that 64% of the infected cows harbored *A. pyogenes*, usually in combination with other bacteria; neither study was designed to determine the incidence of strict anaerobes. In another study, which was designed to determine the incidence of aerobic and strictly anaerobic organisms, 27% of infected cows harbored *A. pyogenes*, and the authors suggested that severe uterine infections may depend on pathogenic synergism between *A. pyogenes* and an anaerobic organism such as *F. necrophorum* (59). Overall, literature indicates that presence of *A. pyogenes* in the uterus is associated with a uterine infection, or impending uterine infection, but absence of *A. pyogenes* does not exclude the possibility that a cow has, or will have, a uterine infection. Indeed, Griffin et al. (28) and Del Vecchio et al. (14) reported that *A. pyogenes* was absent in approximately 35% of the cows with uterine infections, and Paisley et al. (50) suggested that organisms other than *A. pyogenes* and gram-negative anaerobes are responsible for puerperal septic-toxic metritis.

Recent studies indicate clearly that *A. pyogenes*, alone or in combination with other organisms (i.e., *F. necrophorum*, *B. melaninogenicus*, or *E. coli*), can be used to consistently induce uterine infections in puerperal or luteal-phase cows (15, 18, 22). Thus, even though the literature does not support a clear cause and effect relationship between a specific organism or combination of organisms, *A. pyogenes* seems to predispose postpartum cows to uterine infections. The growth of anaerobic bacteria may



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enhance the establishment of *A. pyogenes* and lead to the development of severe uterine infections (18). Indeed, *F. necrophorum* produces a leucotoxin, *B. melaninogenicus* produces a substance that prevents bacterial phagocytosis, and *A. pyogenes* produces a growth factor for *F. necrophorum* (50). The hypothesis that uterine infections depend on a pathogenic synergism between organisms seems plausible (59).

In addition to bacterial risk factors, various management and cow-related risk factors are associated with uterine infections. Cow lifetime records were used to develop equations to predict the incidence of various factors affecting reproductive performance (10). Dystocia, retained placenta, and cow age were significant variables in the equation to predict the incidence of uterine infections; however, the $R^2$ for the model used to develop the equation was .06, indicating that factors not usually included in cow records probably contribute to development of uterine infections (10). Compared with all other cows, younger cows (i.e., 2 to less than 4 yr old) were more likely to have dystocia or assisted deliveries, and older cows (i.e., 7 to less than 10 yr, and at least 10 yr) were most likely to have retained placentas (study conducted in Ontario, Canada; 20). Older cows were 1.58 (7 to less than 10 yr) and 2.49 (at least 10 yr) times as likely to develop metritis as were younger cows (20). In contrast, first-parity Israeli Holstein cows were more likely to develop metritis than were second- through fourth-parity cows, although the risk of retained placenta increased with age of the cow (39).

Twinning and stillbirths were associated with an increased risk of metritis, as were milk fever, ketonuria, and displaced abomasum; although, the associations were not necessarily direct (11, 39). The risk of ketonuria and displaced abomasum increased with

parity (11, 39). Overfeeding (i.e., probably energy) of low milk-yield cows before calving was associated with increased metritis and ketonuria (38). However, the incidence of uterine infections at 40 d postpartum was greater in cows fed a protein-deficient diet than in those fed a protein-adequate diet; the incidence was not different at 25 d postpartum (58). Short heavy first-parity cows were 3.1 times more likely to develop metritis than were short lighter weight or taller cows (40); perhaps short heavy cows were overfed relative to their frame size. Incidence of metritis in one study was greatest in autumn and early winter, but the authors suggested that management factors, rather than season per se, were responsible for the increase (20). Subsequent studies indicated that season did not affect the incidence of metritis (for data and references, see 3). Even though a variety of factors are associated with an increased risk or incidence of uterine infections, studies designed to determine whether a given factor, such as overfeeding of energy, has a direct effect on the incidence of uterine infections are lacking. In fact, because the occurrence and incidence of uterine infections are unpredictable, studies to test specific hypotheses would probably be difficult to conduct in a reasonable period of time.

Because of that difficulty, path analyses have been used to model the relationships among various factors and uterine infections. Odds ratios (OR) for a path are used to estimate risk. For example, the path from parturient paresis to complicated ketosis in one study (12) had an odds ratio of 23.6, which indicates that a cow with parturient paresis was 23.6 times as likely as all other cows in the study to develop complicated ketosis. Curtis et al. (12) modeled the relationship between nutrition and diseases. Based on their final model, retained placenta (OR = 5.7), veterinarian-assisted dystocia (OR = 4.9), and

left-displaced abomasum (OR = 3.6) had direct paths to metritis complex (12). Parturient paresis (OR = 4.0; presumably, prepartum nutritional and metabolic conditions predispose cows to this disease), prepartum dietary protein (OR = .4 and .7; i.e., cows fed greater protein diets had reduced risk, which is consistent with the controlled experiment in 58), days dry (OR = -.02; i.e., shorter dry periods reduced risk), and age of cow (OR = .0005) had direct paths to retained placenta (12). Prepartum dietary energy (OR = .4 and .2) and parturient paresis (OR = 7.2) had direct paths to veterinarian-assisted dystocia (12). The indication that diets with greater energy reduced the risk of dystocia seems contrary to the ideas of Markusfeld (38), but the beneficial effect of shorter dry periods is consistent with data in that report. Uncomplicated ketosis (OR = 11.9) and prepartum dietary energy (OR = .3 and .4) had direct paths to left-displaced abomasum (12). Overall, the study of Curtis et al. (12) indicates that nutritional management and metabolic differences among cows are linked to the occurrence of metritis complex.

Previous data indicated that age affected the incidence of metritis, so Erb et al. (21) developed separate path-analysis models for primiparous and multiparous cows. For primiparous cows, dystocia (OR = 3.0) and retained placenta (OR = 6.5) had direct paths to metritis complex, and dystocia (OR = 4.0) had a direct path to retained placenta (21). For multiparous cows, dystocia (OR = 3.5), retained placenta (OR = 5.8), and parturient paresis (OR = 1.6) had direct paths to metritis complex, and parturient paresis had direct paths (OR = 4.2 and 2.0, respectively) to dystocia and retained placenta.

A more recent study indicates that dystocia (OR = 2.1), retained placenta (OR = 6.0), stillbirth (OR = 1.5), and ketosis (OR = 1.7) have direct paths to metritis complex

(11). Overall, path-analysis and risk-assessment studies indicate consistently that dystocia, retained placenta, and nutritional and(or) metabolic conditions increase the likelihood that a cow will develop metritis. Even though dystocia, retained placenta, and metritis seem to be linked, the reported incidence of metritis is sometimes greater than the incidence of dystocia or retained placenta (17, 21, 39, 41, 63), but 76% of the cows with assisted calving and(or) retained placenta developed metritis (7). Heritability estimates (.19 for first-parity and .26 for second-parity cows) indicate a genetic predisposition for the development of metritis complex (36). Also, genetic correlations between dystocia and metritis (> .8 for first-parity and .28 for second-parity cows) and retained placenta and metritis (.24 for first-parity and > .8 for second-parity cows; 36) were significant.

The cleanliness of a farm, especially the calving area, and hygiene during assisted calvings are believed generally to affect the incidence and outcome of uterine infections (48, 69). The incidence of uterine infections in dairy cows, which often calve in maternity stalls, small dirt and(or) sod lots, etc., is usually greater than that in beef cows, which typically calve on pastures (i.e., considered more sanitary) and receive less assistance during calving (48, 55, 69). Even though a variety of diseases, including uterine infections, seem more common in unsanitary facilities, there is a lack of studies designed to determine experimentally the effect of "cleanliness" on the incidence of uterine infections. Indeed, "cleanliness" would seem difficult to quantify and control. Nevertheless, a recent report indicates that the incidence of endometritis was not different between two hygienically contrasting farms (47). In spite of that report and the lack of experimental data, proper hygiene should be considered an essential component of

livestock management, and the benefits of proper hygiene to animal performance should be taken for granted.

As Curtis et al. (12) indicated clearly, data from path analyses should be used appropriately and cautiously. However, path analyses have provided some of the most useful data available on factors associated with uterine infections. Those factors and associations should be considered clues that could lead to the development of strategies for studying and reducing the incidence of uterine infections. For example, the experimental induction of retained placentas, which are common after corticoid- or $PGF_{2\alpha}$-induced calvings, may be a useful model for studying physiological and pathological changes that lead to uterine infections.

**Consequences**

As with other aspects of uterine infections, the consequences vary considerably among cows and reports. The consequences range from no detectable effect on any measure of productivity, to premature culling, to death of the cow. Fortunately, few cows die from uterine infections. The culling rate in lactations with metritis was 26.6%, whereas it was 20.5% in lactations without metritis; cows with metritis were 1.3 times as likely to be culled as were cows without metritis (3). Metritis complex detected after d 50 postpartum decreased the median productive life of cows by 6 to 8 mo (4).

Apparently, increased culling rates are not a direct effect of metritis. Rather, metritis reduces reproductive performance, and more cows may be culled for poor reproductive performance (42% of cows culled) than for any other reason, including low milk yield (25% of cows culled; 10). Uterine infection was a significant variable in

equations to predict infertility, interval from calving to first recorded estrus (+6.9 d), interval to first service (+7.3 d), interval from first to last service (+15.4 d), and services to conception (+.3; 10). Similarly, metritis increased (+18 d) the interval from calving to conception (3). Abnormal vulvar discharge, presumably an indication of some degree of uterine infection, prolonged the interval from calving to complete cervical involution (49). Interval from calving to conception was greater (+12 to +23 d) and first service conception rate was less (-6 to -21 percentage points) in cows with large (> 55 [primiparous] or 60 [multiparous] mm in diameter) cervices (49). Indeed, most reports seem consistent with data indicating that cows with endometritis after d 21 postpartum are usually less fertile at first service (28, 29).

Path analysis for primiparous cows indicated that metritis was associated with more services to conception, and more services to conception increased the likelihood that a cow would be culled (21). The relationship between metritis and culling was more complex for multiparous cows. Through direct paths, metritis was linked to an increased interval from calving to first service, a reduced likelihood of conception at first service, and an increased incidence of cystic ovaries (21). An increased interval from calving to first service and a reduced likelihood of conception at first service increased the likelihood that a cow would be culled. Cystic ovaries had a direct path to culling and to increased total services; increased total services had a direct path to culling (21). In general, results of path analyses are consistent with numerous observational studies, for which the authors report, for example, the number of days from calving to conception in cows with or without metritis but do not process data further so that the consequences of metritis can

be put into a broader herd-management perspective. That broader perspective is an important advantage of path analyses.

Uterine infections may reduce ovarian follicular development during the early postpartum period. In one study, diameter of the largest follicle during the first 12 d postpartum was less in cows with greater bacterial growth density for uterine fluids, but in another study, there was no significant relationship between follicular diameter and bacterial growth density (51, 52). In addition, the relevance of follicular development during the first 12 d postpartum to overall reproductive performance is not clear. Nevertheless, uterine infections seem to reduce the rate of uterine and cervical involution (23). The diameter of the previously gravid uterine horn was greater than that of the previously nongravid horn, and follicular development was greater in ovaries contralateral to the previously gravid horn (i.e., the smaller horn; 35). The difference in follicular development was evident by d 10 to 15 postpartum, and the contralateral ovary was more likely to have a corpus luteum (35). Also, follicular development was correlated negatively with uterine and cervical diameter (35). Thus, the suggestion of Peter and Bosu (51) seems plausible, but the mechanism is not completely clear.

Many of the financial costs of metritis are indirect, through increased days open, predisposition to other diseases, etc., and difficult for producers to measure. But, an effect of metritis on milk yield can be measured immediately. Metritis detected during d 1 to 21 postpartum reduced average milk yield during d 1 to 5 (-2.6 kg), and cumulative yield for the first 21 (-50.1 kg), 22 to 49 (-62.0 kg), and first 119 d (-265.8 kg) of lactation (13). Metritis detected during d 22 to 49 or 50 to 119 postpartum had no

significant effect on milk yield during any of the periods evaluated over the first 119 d of lactation (13). As Deluyker et al. (13) pointed out, metritis in other studies did not affect milk yield. However, some pharmaceuticals used to treat uterine infections leave residues in milk (37), the milk must be discarded, so the yield of marketable milk can be reduced (3).

Consequences of uterine infections are varied and difficult to predict. Magnitude of the consequences should be expected to vary with severity of infection, time postpartum, herd health practices, etc. One should expect uterine infections to reduce reproductive performance, increase culling rate, and have a negative financial impact on a dairy operation (3).

**Treatment and Prevention**

Treatments for endometritis and metritis are controversial, and an in-depth description is beyond the scope of this review. Intrauterine infusion of various antimicrobial-antibacterial compounds is a traditional treatment, but, based on current evidence, intrauterine infusions do not seem efficacious and may be harmful (26, 34, 50, 55, 65). Plus, intrauterine antimicrobial-antibacterial compounds can contaminate milk and force it to be discarded (26, 37). Draining uterine fluids from cows with acute puerperal metritis is a common, but apparently an ill-advised, procedure; manipulation of an infected uterus can exacerbate the problem (26). Some authors suggest that the type of treatment has little effect on the outcome of puerperal metritis, and the goal should be to prevent life-threatening changes and administer supportive therapy while the uterus

"heals" itself (55). Similarly, the economic benefit of conventional treatments for endometritis has been questioned (6).

Treatment of pyometra (sometimes called, chronic endometritis) does not seem controversial. Authors agree that $PGF_{2\alpha}$, or an analogue, is the most appropriate treatment (26, 50). By most definitions, pyometra is confined to cows with active corpora lutea, so $PGF_{2\alpha}$ causes luteolysis and permits the uterus to resolve the infection (26, 50, 69). Also, $PGF_{2\alpha}$ may be an effective treatment for endometritis; it certainly seems as effective as other treatments, it is the least harmful, and milk does not have to be discarded (6, 26, 44, 50).

Preventing uterine infections is difficult, because the causes cannot be defined clearly. Proper attention to facilities maintenance and sanitation, and periparturient hygiene may be the best defense. A number of investigators have evaluated the usefulness of routine intrauterine treatment of postpartum cows for preventing uterine infections (for an example, see 45). However, routine intrauterine treatments do not seem efficacious, and they are not currently recommended (69). In spite of that, there are continued efforts to develop routine prophylactic intrauterine treatments (16).

**Mechanistic Considerations**

A central question is, how can most postpartum cows manage bacterial contamination without developing uterine infections that impair reproductive performance, while other cows in the same herd, with presumably the same general management, develop severe uterine infections that reduce fertility? The competence of the immune system may be the major difference between the two types of cows. A number of authors

have suggested that the function of neutrophils is impaired in cows that develop uterine infections (for reviews, see 8, 33, 34).

Neutrophils are phagocytic leukocytes that are involved in nonspecific (i.e., phagocytosis of "new" pathogens) and specific (i.e., phagocytosis of antibody-opsonized pathogens) immune functions. Nonspecific phagocytosis seems to be an animal's initial defense against invading pathogens (33). As described in Hussain (33) and Cai et al. (8), the effectiveness of neutrophils depends on their ability to move to the site of infection and destroy the invading pathogen. A brief, but rather simplistic, description of that process may help put the importance of properly functioning neutrophils into a perspective relevant to uterine infections; thorough descriptions are available in physiology and immunology textbooks.

When pathogens invade and cause inflammation, chemotactic agents "call" neutrophils, and other elements, from blood to the site of infection. The chemotactic process provides neutrophils with a chemical gradient to follow and is different from undirected random movement of neutrophils to the site of infection. After neutrophils arrive, they must adhere to and ingest the pathogens. The ingested pathogen becomes enclosed in a vacuole within the cytoplasm of the neutrophil, and the pathogen is digested and destroyed. Variables associated with the major aspects of neutrophil movement and pathogen destruction can be quantified and related to the predisposition to and ability to recover from uterine infections (8). In addition, pathogens may induce the development of active immunity, which involves B and T lymphocytes, and the ability of lymphocytes to divide (blastogenesis) can be used as a measure of immune function.

In clinically normal cows, the number of peripheral neutrophils increased during the last 10 to 15 d of pregnancy, then decreased during the first 7 d after calving (8, 60). The phagocytic activity of neutrophils increased prepartum, decreased sharply at calving, and then increased steadily for the first 14 d postpartum (60). In cows that subsequently developed metritis, the prepartum increase in number of neutrophils was attenuated, and the postpartum decrease was accentuated (8). Neutrophil function also was reduced before and after calving in cows that developed metritis (see 8, 33). Phagocytic activity was reduced in cows that retained placentas, which is a predisposing factor for metritis, and in cows that developed metritis but did not retain placentas (see 33). Random movement of neutrophils, but not chemotaxis, measures related to pathogen digestion, and antibody-dependent cell-mediated cytotoxicity were reduced in cows that developed metritis, and there was a significant correlation between random migration and phagocytosis (8).

Uterine trauma, such as dystocia, manual removal of retained placentas, and intrauterine infusions, reduced uterine and blood neutrophil phagocytic activity (33, 50). In addition, even though *A. pyogenes* did not affect chemotaxis, *Bacteroides* ssp., which is often associated with *A. pyogenes* uterine infections, decreased neutrophil chemotaxis and phagocytosis in vitro (67). Phagocytic activity of neutrophils in peripheral blood and uterine lumen were similar, although somewhat less for uterine lumen, and activity in peripheral blood may be a gross estimate of uterine neutrophil function (1). Overall, data indicate that neutrophil function in peripheral blood may be impaired before calving in some cows, this impairment may predispose cows to postpartum uterine infections, and

organisms associated with uterine infections may further impair phagocytic activity and neutrophil movement to the site of infection. Continued impairment of neutrophil function after calving may further increase the likelihood that a cow will develop a severe uterine infection.

Blastogenesis of peripheral blood lymphocytes in response to mitogens decreased during the last 21 d of pregnancy and increased during the first 14 d postpartum in clinically normal cows (60). However, blastogenesis of peripheral blood lymphocytes in response to mitogens was less for cows with difficult parturition than for cows with normal parturition (42). Perhaps some aspect of difficult parturition impairs subsequent humoral and cell-mediated immunity.

A recent study provides evidence that intrauterine inoculation of heifers with *A. pyogenes* may produce some measure of active immunity (68). Other studies indicate that multiparous cows may be more resistant to *A. pyogenes* infections than are primiparous cows (33). Perhaps the uterine immune system in older cows can recognize *A. pyogenes* and mount a humoral and cell-mediated defense against the organism. This raises the intriguing possibility of intrauterine inoculations to reduce the incidence of uterine infections. However, because the immune system may be impaired prepartum in cattle that subsequently develop uterine infections, it seems to be impaired in cows with uterine infections, and the response to intrauterine inoculation seemed sluggish, the efficacy of intrauterine inoculations may be questionable. But, the possibility should not be ignored.

Several studies with cattle indicate that hormonal environment affects immune function. A progesterone-dominated environment typically down regulates immune

function, and an estrogen-dominated environment, under most circumstances, is associated with up-regulated immune function (30). Cattle are more susceptible to uterine infections during diestrus than during estrus (5, 57), and leukocytic response to intrauterine bacterial inoculation was less during diestrus than during estrus (9, 30). In sheep, percentage of vena caval lymphocytes and basal and mitogen-stimulated activity of B and T lymphocytes were greater during estrus than during diestrus (56). Estrus, but not diestrus, ewes resisted uterine infections (56).

Concentrations of estradiol-17β increase considerably during the last 10 to 15 d of pregnancy in cattle, but progesterone concentrations decrease only modestly during most of the same period; progesterone decreases abruptly just before calving, and estradiol decreases abruptly just after calving (e.g., 61). Changes in immune function are consistent with changes in estradiol and progesterone concentrations around calving and during the estrous cycle of cattle and sheep (8, 30, 56, 60). Perhaps the increase in estradiol and abrupt decrease in progesterone permit the immune system to up regulate at times when bacteria are likely to invade the reproductive tract. Basal concentrations of progesterone during the postpartum period may leave the immune system somewhat up regulated and permit most cows to clear intrauterine bacteria before they can proliferate out of control. In fact, we were unable to induce uterine infections in clinically normal postpartum beef cows until after they had developed corpora lutea (15). This relationship between ovarian hormone environment and immune function seems to explain why pyometra is only detected when cows have active corpora lutea. Also, the relationship seems to explain why $PGF_{2\alpha}$ has become the treatment of choice for pyometra; $PGF_{2\alpha}$ induces luteolysis,

estradiol concentrations increase, immune function up regulates, and the uterus is able to clear the infection.

Arachidonic acid metabolites also change considerably during the peripartum period. The postpartum pattern of decrease in 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM) concentrations are closely associated with the pattern of uterine involution in clinically normal cows, and an altered pattern of PGFM is associated with induced and spontaneous endometritis in cows (14, 15, 35). In vitro experiments indicate that $PGF_{2\alpha}$, leukotriene $B_4$, 5-hydroxyeicosatetraenoic acid (HETE), 15-HETE, and lipoxin $B_4$ were chemoattractant to neutrophils (31). Perhaps $PGF_{2\alpha}$ has another role (i.e., in addition to inducing luteolysis) in resolving pyometra. We found in a recent study with sheep that exogenous $PGF_{2\alpha}$ enhances uteroovarian release of $PGF_{2\alpha}$ (66); increased uterine $PGF_{2\alpha}$ could enhance neutrophil movement to the uterus. In contrast to the effects of $PGF_{2\alpha}$, intrauterine $PGE_2$ suppressed blastogenesis of peripheral lymphocytes, reduced intrauterine immunoglobulin concentrations, reduced the rate of uterine involution, and increased the incidence and severity of uterine infections in cows (62). Prostaglandins and other arachidonic acid metabolites may be important mediators of resistance or susceptibility to uterine infections.

Gram-negative organisms, which are associated with some types of uterine infections, produce endotoxins (24). Endotoxins can escape from the uterus, enter the systemic circulation, increase cortisol production, and suppress the preovulatory LH

release in heifers (53, 54). The authors speculated that endotoxins are related to the increased incidence of cystic ovaries in cows with uterine infections.

## CONCLUSIONS

Uterine infections have negative effects on various measures of productivity of dairy cows. The extent of the economic impact of uterine infections is often difficult to measure, but the impact should not be considered trivial. Postpartum cows typically develop mild endometritis, but most cows are able to clear pathogenic organisms that cause endometritis before any measure of productivity is affected. Aberrant immune function before and after calving seems to predispose some cows to severe uterine infections that reduce fertility and other measures of productivity. Thus, methods for regulating immune function in periparturient cows would seem to have tremendous potential for preventing and(or) treating uterine infections. For now, proper attention to facilities maintenance and sanitation, and periparturient hygiene, especially during assisted calving, may be the best defense.

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