

# Effects of Intrauterine Infusions of Dilute Lugols Solution on the Bovine Estrous Cycle

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In cattle the uterus determines the lifespan of the corpus luteum. Hysterectomy (8) prolonged the life of the corpus luteum while uterine distention from balloons (3) and plastic coils (1) and irritation from contaminated seminal fluid (3), a viral agent (4), and iodine solutions (5,6,9) all shortened the estrous cycle.

These substances in the uterus activate a uterine luteolytic mechanism. An understanding of uterine luteolytic mechanisms may permit the development of both satisfactory estrous synchronization techniques and contraceptive procedures. The objective of this study was to determine the effects of intrauterine infusions at various stages of the estrous cycle on estrous cycle length, estrous behavior, ovarian activity, and levels of luteinizing hormone (LH) in peripheral plasma of cattle.

## Material and Methods

Data for this study were collected from 44 cycles in 28 primiparous Holstein cows. The cows had at least one normal estrous cycle prior to the uterine infusion and were randomly allocated at estrus to one of the following treatment groups:

Treatment Group	Intrauterine Treatment	Day of Estrous Cycle for Infusion
I	2% Lugols in 250 ml of Physiological Saline	1
II	2% Lugols in 250 ml of Physiological Saline	4
III	2% Lugols in 250 ml of Physiological Saline	11
IV	250 ml of Physiological Saline	1
V	250 ml of Physiological Saline	4
VI	Control	—

These cows were kept in loose housing and milked in a milking parlor. They were fed grain, hay and corn silage to meet production requirements.

The preproductive tracts of these cows were palpated *per rectum* at least twice weekly, starting

at seven days postpartum. The following data were recorded at each observation and examination: 1) color of vulva and nature of discharge; 2) diameter of cervix; 3) diameter of each uterine horn at the bifurcation of the horns; and 4) location and size of follicles and corpora lutea on both ovaries. This information was used to verify estrus and ovulation. All cows were observed twice daily for 20 minutes to detect signs of estrus.

Each cow was bled from the jugular vein at estrus (day 0) and on days 2, 4, 7, 11, 18, and 20 and daily until estrus. Bleeding was discontinued in Group II at estrus about six days after infusion. Blood serum luteinizing hormone (LH) was quantified by the double antibody radioimmunoassay procedure (7).

## Definitions

Estrus was characterized by the presence of a mature follicle (15-20 mm in diameter) in conjunction with increased uterine tone, hyperemia and edema of the vulva, and a mucous discharge from the vulva.

Standing estrus was considered to have occurred when the cow stood to be mounted. It was recorded at the time of the initial observation regardless of the duration of estrus.

Silent estrus was considered to have occurred when no behavioral signs of standing estrus were observed, even though the other signs were present. It was recorded as occurring one day prior to ovulation as detected by palpation *per rectum*.

An estrous cycle was the period measured in days between a consecutive silent or standing estrus and ovulation.

A cystic follicle was a follicle that was greater than 2.5 cm in diameter on three successive examinations over a 10-day period.

A cystic corpus luteum was defined as a corpus luteum which developed after ovulation and contained a fluid-filled central cavity, approximately 10 mm or more in diameter. A smaller

Table 1  
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Criteria	Lugols Day 1 (Group I)	Lugols Day 4 (Group II)	Lugols Day 11 (Group III)	Saline Day 1 (Group IV)	Saline Day 4 (Group V)	Control (Group VI)	Mean
Number of Cows	7	10	6	5	8	8	
Postpartum Interval (Days)	57	62	56	40	58	46	54
Estrous Cycle Length (Days)							
Infusion Cycle	22.0	10.3	21.3	20.2	20.9	21.3	18.7
Subsequent Cycle	23.0	21.3	22.0	20.0	19.0	20.3	21.0
Standing Estrus (%)							
Infusion Cycle	85	70	50	80	50	100	74
Subsequent Cycle	100	100	67	50	50	67	70
Ovarian Function							
Corpora Lutea (%)							
Infusion Cycle	71	80	80	100	80	63	77
Subsequent Cycle	67	100	100	100	80	83	86
Cystic Corpora Lutea (%)							
Infusion Cycle	29	20	20	0	20	37	23
Subsequent Cycle	33	0	0	0	20	17	14
Cystic Follicle (%)							
Infusion Cycle	0	0	0	0	0	0	0
Subsequent Cycle	0	0	0	0	0	0	0

cavity was not usually detectable by palpation *per rectum*.

#### Results and Discussion

The mean interval from parturition to intrauterine infusion was 54 days (Table 1). The infusion of 250 ml of 2% Lugols solution into the uterus on days 1, 4, or 11 (Table 1) of the estrous cycle resulted in estrous cycles of 22.0, 10.3, and 21.3 days, respectively, while infusion of 250 ml of saline on days 1 and 4 resulted in cycles of 20.2 and 20.9 days respectively ( $P < 0.01$ ). In other words, the only one of five intrauterine infusion treatments which altered estrous cycle length was the infusion of 250 ml of 2% Lugols on day 4. Similar results have been found in other studies when the uterus was infused on days 3 to 5 of the cycle (5,6,9). The infusion of 40 ml of 0.2% nitrofurazone solution on day 3 of the estrous cycle also caused an early return to estrus (2).

When 250 ml of 2% Lugols was infused into the uterus on day 4 (Group II), estrus and ovulation occurred in 6.3 days for a 10.3 day estrous cycle, while estrus began 16.9 days after infusion of 250 ml of saline only on day 4 (Group III) for a normal estrous cycle of 20.9 days. Since the same volume of material was infused in both groups of cows, the short cycle must be attributed to the effect of the Lugols on the uterus. The iodine solution produces a severe irritation of the endometrium as revealed by endometrial biopsy, resulting in necrosis and sloughing of the

endometrial surface. This material in the lumen of the uterine horn resembles a purulent exudate.

In one study the estrous cycle was shortened by the infusion of only 0.1 ml of iodine solution into the ipsilateral uterine horn, which caused irritation to a 3 to 4 cm (2) area of the endometrium (5). These data also confirm that uterine irritation is more important than distention in altering the estrous cycle.

The uterus normally regulates the estrous cycle by the production of a luteolytic factor. Evidently this mechanism was activated following infusion of an irritant on day 4 of the estrous cycle.

The intrauterine infusion of antibiotics and antiseptics is routinely used after calving for metritis and after insemination in cows with a history of repeat breeding. The results of this study indicate that treatments in the repeat breeder should be administered no later than two days after breeding to avoid luteolysis, embryonic death and an early return to estrus. The ovaries should also be palpated at the time of the postbreeding infusion to document ovulation.

The subsequent cycles in all treatment groups were normal indicating that there was no latent effect from the intrauterine infusions (Table 1). These results have been confirmed by similar findings in other studies (5,6,9).

Estrous behavior was observed and the occurrence of standing estrus reported. Standing

and silent (unobserved) estrus was verified by palpation *per rectum* to document ovulation and the development of a corpus luteum. Standing estrus occurred in 74% of all cows during the infusion cycle and in 70% during the subsequent cycle. The differences between treatments and controls were not significant ( $P > 0.05$ ), indicating that the distention and irritation of the uterus did not affect estrous behavior during either the infusion or subsequent cycle.

Ovarian activity was measured by the development of corpora lutea, cystic corpora lutea, and cystic follicles. The mean occurrence for corpora lutea was 77% during the infusion cycle and 86% during the subsequent cycle (Table 1) while cystic corpora lutea occurred in 23% and 14% of the infusion and subsequent cycles respectively (Table 1). No cystic follicles were found during the two cycles studied. The differences in ovarian activity as measured by the occurrence of corpora lutea, cystic corpora lutea, and cystic follicles between infused and control groups were not significant ( $P > 0.05$ ), indicating that the irritation and distention of the uterus did not affect these factors during either the infused or subsequent cycles.

The growth and maintenance of the corpus luteum or cystic corpus luteum was adversely affected by the intrauterine infusion of saline and Lugols on day 4 of the estrous cycle. In this group the corpus luteum or cystic corpus luteum continued to increase in size until day 7 or 8, followed by rapid regression and follicular development which resulted in estrus on day 10 or 11. The decline of the corpus luteum and follicle growth was similar to that observed just prior to estrus during a normal cycle in the other treatment groups. The delay in luteal regression following the infusion of an endometrial irritant suggests that the infusion destroyed rather than released the luteolytic factor, with production dependent upon endometrial repair.

Peripheral plasma levels of LH declined from 12 ng/ml at estrus to approximately 1 ng/ml during the balance of the cycle in all groups. A surge in LH occurred again in all groups at the onset of the next estrus. The levels of LH varied with the stage of the estrous cycle. The LH surge which occurs for six to eight hours near onset of estrus was sometimes missed by daily bleeding.

It was concluded from this study of 44 cycles in 28 primiparous Holsteincows that the infusion of the uterus with 250 ml of 2% Lugols in saline on day 4 of the estrous cycle caused luteolysis and a return to estrus at approximately day 10. Since the infusion of saline at this time resulted in a normal

and saline on day 4 had no effect on the estrous cycle. All infusions had no effect on subsequent cycles.

The infusions on days 1, 4, and 11 of the cycle did not affect behavior at estrus or the occurrence of corpora lutea, cystic corpora lutea, or cystic follicles during the treated and subsequent cycles. The levels of LH in peripheral plasma varied with the stage of the estrous cycle in all groups.

All infusion in repeat breeder cows should be performed on the first or second day after estrus to avoid cycle alterations due to luteolysis resulting in embryonic mortality and a premature return to estrus. When mismating has occurred, a therapeutic abortion can be performed by infusing the uterus with an irritating substance such as 2% Lugols in saline at approximately day 4. Estrous synchronization can also be facilitated in the same manner at this time.

#### Summary

Estrous cycle length, estrous behavior, ovarian activity, and peripheral plasma luteinizing hormone (LH) were determined after intrauterine infusions of 250 ml of 2% Lugols\* in Physiological Saline\*\*. This study was conducted during 44 cycles in 28 Holstein cows between 30 and 90 days postpartum. They were randomly allotted after at least one normal estrous cycle to control and treatment groups which received intrauterine infusions of 250 ml of 2% Lugols in saline on days 1, 4, or 11 of the estrous cycle (estrus = day 0), or 250 ml of saline on days 1 or 4 of the estrous cycle. Twice weekly palpations *per rectum* were performed. Lugols infusion at day 4 reduced the estrous cycle length of 10.3 days from 21.3 days ( $P < 0.01$ ) in controls and affected luteal function but not the occurrence of standing estrus. Lugols infusions at days 1 and 11 and saline infusions at days 1, 4, and 11 did not affect estrous cycle length, the occurrence of standing estrus, or ovarian activity. Estrous cycles subsequent to treatment were normal. A surge in peripheral levels of LH occurred near the onset of estrus in all groups.

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\*Lugol's Solution, Humco Laboratory, Texarkana, Texas.

\*\*Physiological Saline Solution, Haver-Lockhart Laboratories, Shawnee, Kansas.

nation with Semen Contaminated with IBR-IPV Virus. *Cornell Vet.* 57:3, 1967. — 5. Nakara, T., I. Domeki, and M. Yamauchi. Local Effect of Intrauterine Injection of Iodine Solution on the Lifespan of the Corpus Luteum of the Cow. *J. Reprod. Fertil.* 26:423, 1971. — 6. Nakara, T., I. Domeki, and M. Yamauchi. Effects of Intrauterine Injection of Iodine Solution on the Estrous Cycle Length of the Cow. *Nat. Inst. Hlth. Quart.* 11:211, 1971. — 7. Niswender, G. D., L. E. Reichert, Jr., A. R. Midgley, Jr., and A. V. Nalbandov. Radioimmunoassay for Bovine and Ovine Luteinizing Hormone. *Endocrinol.* 84:1166, 1969. — 8. Wiltbank, J. N. and L. E. Casida. Alteration of Ovarian Activity by Hysterectomy. *J. Animal Sci.* 15:134, 1956. — 9. Yamauchi, M., T. Nakahara, Y. Kaneda, and S. Inui. Effects of Uterine Distention on the Oestrous Cycle of the Cow. *J. Reprod. Fertil.* 13:379, 1967.

#### QUESTIONS AND ANSWERS

**Dr. Zemjanis:** I would just like to indicate that I agree with your findings but at least with our research results, saline did not have any effect on shortening the cycle.

**Dr. Morrow:** Well, there is one study by Guenther in Germany where he has done it on day four. He did not have any early return to estrus but Nicar's data pretty much agrees with what we found or we agree with what he has found. There is also the study of Guenther's that was published this summer where on day three, using infusions in seven animals, he had an average interval to estrus of 15 days. However, there was quite a lot of variation in his particular study.

**From the Floor:** Did you have any problem inserting these?

**Dr. Morrow:** I would say at the time we were using them in this particular study, no, we did not. We are talking about day 20 up to day 25 and as long as day 30 on a couple of animals so we did not have a particular problem. Certainly in a clinical situation where you may be getting outside of this particular area, then you can run into some problems, and we have had particular animals where we were unable to insert them. So, generally speaking, around three weeks as I have indicated—you have to wait until the cervix contracts down so it is small enough to hold the device and yet not too small to get it in.

**Question:** What antibiotics are the least irritating?

**Dr. Morrow:** Looking at various antibiotic infusions, my impression would have been that if any would have had little effect, furacin would have been one of those, but as I say, Dr. Guenther is finding that it is also having a shortening effect on the estrus cycle so I guess other than saline, I would not be able to say at this point because we have not tested a number of different ones. I think there is one other comment which I should make and that is that we used about 30 of these in this particular experiment. We did have one animal where when we went to remove this UIU the catheter broke off and the UIU remained in the body of the uterus!

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## Applying Research in Herd Reproductive Service

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Major advances in our knowledge of bovine reproduction in the past 25 years have occurred in the fields of pathology, physiology, endocrinology, microbiology, nutrition and zootechnics. These have resulted in an increased understanding of puberty; the estrous cycle; ovulation; C.L. formation, maintenance and involution; pregnancy; parturition; uterine involution; sperm cell development; accessory gland function; copulation; ejaculation; sperm transport; fertilization and ova transplant. Radioimmunoassays and protein-binding assays that can readily determine the blood levels of progesterone, estrogen, testosterone, corticosteroids, LH, and other proteinaceous or steroid hormones have rapidly forwarded research in endocrinology.

Other advances in basic and applied research have revealed the nature, complexity, and impor-

tance of external stimuli such as light, stress, nutrition and visual, auditory, olfactory, physical and endocrine factors on the central nervous system to cause the manifestation of estrus as well as the production of at least nine distinct polypeptides or releasing factors from centers in the hypothalamus (2). When these factors pass to the anterior pituitary gland by means of the hypophysical portal circulation, they regulate the release of the many endocrine substances in that gland. Preliminary research indicates that the gonadotropic releasing factor (GnRF) that releases FSH and LH from the pituitary gland is of value in the treatment of cystic ovaries (3). It may possibly prove of value in the treatment of certain types of anestrus. Japanese and other studies have shown that parenteral injection of heterologous proteinaceous anterior pituitary hormones such as