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PROGESTERONE AND 15-KETO-13,14-DIHYDROPROSTAGLANDIN $F_{2\alpha}$ LEVELS IN PERIPHERAL CIRCULATION AFTER INTRAUTERINE IODINE INFUSIONS IN COWS*

By

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KINDAHL, H., E. GRANSTRÖM, L.-E. EDQVIST, B. GUSTAFSSON, G. ÅSTRÖM and G. STABENFELDT: *Progesterone and 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ levels in peripheral circulation after intrauterine iodine infusions in cows.* Acta vet. scand. 1977, *18*, 274—286. — The effect of intrauterine iodine infusion on estrous cycle length was studied in four cows. The infusions were performed at various times of the estrous cycle: early, middle, late, and during luteolysis. Blood samples were drawn every third hour from the jugular vein. Progesterone and 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ (the main metabolite of $PGF_{2\alpha}$) were measured to monitor luteal activity and prostaglandin release. No release of prostaglandins was observed immediately following intrauterine infusion. Infusion in two cows on day 5 of the estrous cycle resulted in prostaglandin release after 54 and 69 hrs., respectively, followed by luteal regression and the occurrence of estrus at approx. five days after infusion. Infusions performed on days 11 or 12 resulted in prostaglandin release after 147 and 120 hrs., respectively, followed by luteolysis and heat after a 19 day estrous cycle. Infusion in two cows at days 16 and 17 resulted in prostaglandin release after 117 hrs. in both animals. One cycle was prolonged whereas the other cycle was normal in duration. One cow

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infused on day 20 following the occurrence of the first prostaglandin surge had a cycle length of 26 days, whereas another cow infused on day 20 was not affected because luteolysis was essentially complete by the time of infusion. One animal infused on day 5 did not respond to the iodine infusion. In this animal, however, the corpus luteum was not completely developed prior to the infusion.

From this study it can be concluded: 1) intrauterine iodine infusions performed after the development of a progesterone secreting corpus luteum result in prostaglandin release within three to six days with the subsequent occurrence of luteolysis; 2) luteolysis was in all cases observed in connection with prostaglandin $F_{2\alpha}$ release of the same order of magnitude and duration as during normal luteolysis.

prostaglandin release; progesterone; cow; estrous cycle; iodine infusion.

Wiltbank & Casida (1956) showed that the uterus is necessary for the cyclic regression of the corpus luteum in the cow. That the uterine luteolysin is most likely prostaglandin $F_{2\alpha}$ has been shown by exogenous administration of the compound (*Rowson et al.* 1972) as well as by measurement of $PGF_{2\alpha}$ in the utero-ovarian vein (*Nancarrow et al.* 1973) or the uterine vein (*Shemesh & Hansel* 1975). *Hixon & Hansel* (1974) have shown that a preferential transfer of $PGF_{2\alpha}$ exists between the uterus and the ovarian artery in the cow. The released $PGF_{2\alpha}$ is rapidly metabolized to 15-keto-13,14-dihydro-$PGF_{2\alpha}$ in the bovine (*Kindahl et al.* 1976 a, b). Measurement of this metabolite in the peripheral circulation has provided a reliable method of estimating the release of primary $PGF_{2\alpha}$ (*Kindahl et al.* 1976 b). Recently, several studies have described the peripheral blood levels of this prostaglandin metabolite during the bovine estrous cycle including the time of luteolysis (*Peterson et al.* 1975, *Kindahl et al.* 1976 a, b). The prostaglandin release occurs normally over two-three days as rapid pulses with durations of 1—5 hrs. prior to and during luteolysis (*Kindahl et al.* 1976 a).

Several studies have demonstrated the effect of intrauterine iodine infusions, a commonly used method for treatment of subclinical endometritis in cows, on estrous cycle length in the bovine species (*Nakahara et al.* 1967, 1971, 1975, *Morrow et al.* 1971, *Grunert et al.* 1973, *Seguin et al.* 1974, *Domeki et al.* 1975). In general, uterine infusions performed early in the estrous cycle shortened the cycle length and infusions late in the cycle lengthened it, while infusions during mid-cycle appeared to have no effect. Iodine is rapidly absorbed from the uterine cavity (*Ekman et al.* 1965) and could influence endocrine organs; however, it has recently been suggested that the irritating effect of

iodine on the endometrium is the important factor in altering the cycle length (*Seguin et al.*). Thus, during endometrial repair, $PGF_{2\alpha}$ might be synthesized and induce luteolysis.

The aim of the present investigation was to study the effect of intrauterine iodine infusions on prostaglandin release by measurement of the main prostaglandin $F_{2\alpha}$ plasma metabolite, 15-keto-13,14-dihydro-$PGF_{2\alpha}$ and on the function of corpus luteum as determined by measurement of progesterone.

MATERIALS AND METHODS

Four cows of the Swedish Red and White Breed (SRB) between three and four years of age were used. The animals were held in stanchions for several months prior to and during the experiments. They were observed daily for external signs of heat with more extensive clinical examinations performed two-three times weekly. These examinations included palpation per rectum of the uterus and ovaries as well as inspection of the vaginal and vestibular mucosa and the external os of the cervix through a glass tube speculum. The day of heat (= day 1 of the estrous cycle) was established by vaginal inspection as well as the estrous behaviour of the animals. Only vaginal examinations were performed during treatment cycles and, in these cases, blood samples were collected prior to the examination. All cows appeared normal during the entire experimental period of several months.

The four animals were designated nos. 1—4, and the length of control estrous cycles for each animal was: no. 1: 22.4 ± 0.5 days (mean ± standard deviation of nine estrous cycles); no. 2: 22.6 ± 0.5 days (11 estrous cycles); no. 3: 23 and 22 days; and no. 4: 21 and 22 days (two cycles each).

The treatment consisted of intrauterine infusion of 200 ml of an iodine solution (1 % Lugol's solution ($I:KI:H_2O = 5:8:87$) in 0.9 % saline) through a plastic intrauterine catheter.

In order to study the immediate effect of iodine infusion, two cows were infused on day 5 and blood samples were obtained every hour for 10 hrs. No more samples were collected from these animals during these cycles. The rest of the animals were studied at 3 hr. intervals (range 4—11 days) following infusion at various time of the estrous cycle: early (days 5, 5, 5), middle (days 11, 12), late (days 16, 17) and during luteolysis (days 20, 20) (Table 1). As each animal was treated more than once,

the animals were allowed at least one recovery cycle between two treatment cycles. The blood samples were collected by puncture of the jugular vein in heparinized tubes (Vacutainer® System, Becton-Dickinson). The tubes were immediately centrifuged and the plasma was removed and stored in plastic tubes at —20°C until analyzed.

Plasma samples with progesterone levels exceeding 1 ng/ml were analyzed by a competitive protein binding technique (*Edqvist et al.* 1970). Progesterone levels below 1 ng/ml were analyzed by a radioimmunoassay method (*Kindahl et al.* 1976 a, *Bosu et al.* 1976). The prostaglandin release was followed by measurement of the 15-keto-13,14-dihydro-$PGF_{2\alpha}$ with radioimmunoassay (*Kindahl et al.* 1976 a, *Granström & Kindahl* 1976).

RESULTS AND DISCUSSION

The results are shown in Figs. 1—4 and are compiled in Table 1. When infusion was performed on day 5, the interval from intrauterine iodine infusion to prostaglandin release was approx. 60 hrs., in contrast to about 120 hrs. when infusion was done later (between days 11 and 17) of the cycle. Two animals

Table 1. The effect of intrauterine iodine infusion on the prostaglandin release and the length of the estrous cycle.

Day of infusion	Cow no.	Pretreatment cycle (days)	Treatment cycle (days)	Posttreatment cycle (days)	Interval from infusion to PG release (hours)	Interval from infusion to estrus (days)
Early						
5	1	22	10	22	N.D.	5
5	2	23	10	22	N.D.	5
5	1	23	9	23	54	4
5	2	23	10	22	69	5
5	3	22	23	21	33	18
Middle						
11	1	23	19	22	147	8
12	2	22	19	23	120	7
Late						
16	1	22	23	23	117	7
17	2	22	25	23	117	8
20	4	21	26	22	93	6
20	3	23	21	N.D.	—	—

N.D. = Not determined.

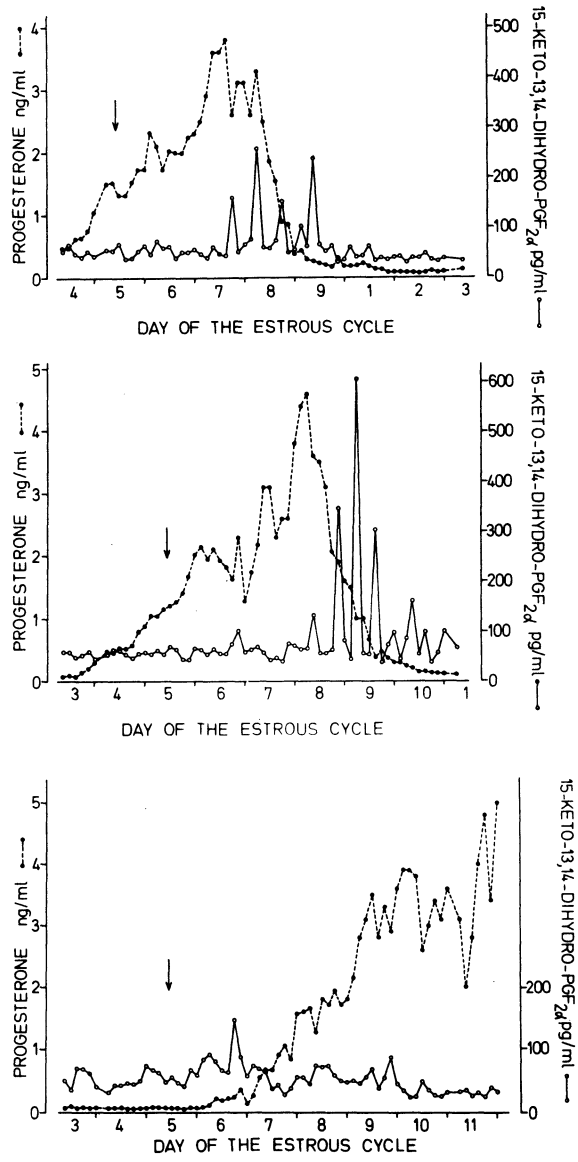

Figure 1. Peripheral plasma levels of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (o———o) and progesterone (•·····•) in cows no. 1 (upper panel), no. 2 (middle panel) and no. 3 (lower panel) subjected to intrauterine iodine infusion on day 5 of the estrous cycle. Arrows denote time of infusion.

were treated with iodine around the time of expected luteolysis: one animal was infused after several prostaglandin metabolite peaks had occurred and luteolysis was almost complete, and the other cow after one minor peak of the prostaglandin metabolite. In the first animal no effect on the cycle length could be seen, however, the second animal had a cycle length around 3.5 days longer than its control cycles.

One additional animal was treated on day 5 (cow no. 3, Fig. 1, lower panel) at a time at which progesterone levels were very low, approx. 100 pg/ml. Only one significant prostaglandin metabolite peak was observed 33 hrs. after the infusion, and subsequent regression of the corpus luteum did not occur. A retrospective study of the clinical records of this cow revealed that the corpus luteum routinely could not be palpated until day 7 or 8, suggesting a slow development of the corpus luteum. Progesterone analysis of daily samples obtained earlier from the cow revealed low progesterone values around day 5 and an increase from around day 7. The results from this cow suggest that the endometrium may require a minimum exposure time to progesterone, before $PGF_{2\alpha}$ synthesis and release can appear. This is in agreement with earlier reported findings in the ewe (*Roberts et al.* 1975).

Ovulation occurred at the expected time after estrus in all cycles with induced heat.

It is obvious that the estrous cycle of the cow can be manipulated through intrauterine infusion of an irritant, presumably because of the release of $PGF_{2\alpha}$. It is apparent from this study that $PGF_{2\alpha}$ in the cow is not released immediately after infusion of the irritating iodine solution, because cows nos. 1 and 2, which were sampled hourly for 10 hrs. after one infusion, showed no increase in the prostaglandin metabolite levels during the immediate observation period. However, both these animals showed heat 5 days after treatment (Table 1). Furthermore, in the remaining experiments, where samples were collected with 3 hr. intervals, increases in the prostaglandin metabolite level were never seen during the first few days after the infusion.

The delay in release of prostaglandin is likely to account for the different effects of intrauterine iodine infusion on estrous cycle length that has been reported by a number of authors (for references see the Introduction). Estrous cycles will be shortened if the induced prostaglandin release occurs prior to the normally

expected time, i.e. about day 18 or 19 of a 21 day cycle. Because of the five days' delay in prostaglandin release that follows iodine infusion during mid-cycle, infusion must be done before day 12 or 13 if the cycle is to be shortened. The two cows infused on days 11 and 12 of the cycle had each a treatment cycle length of 19 days, which was shorter than their control cycles. These results are in contrast to earlier reports, which indicated that intrauterine infusion of iodine at mid-cycle had no effect on

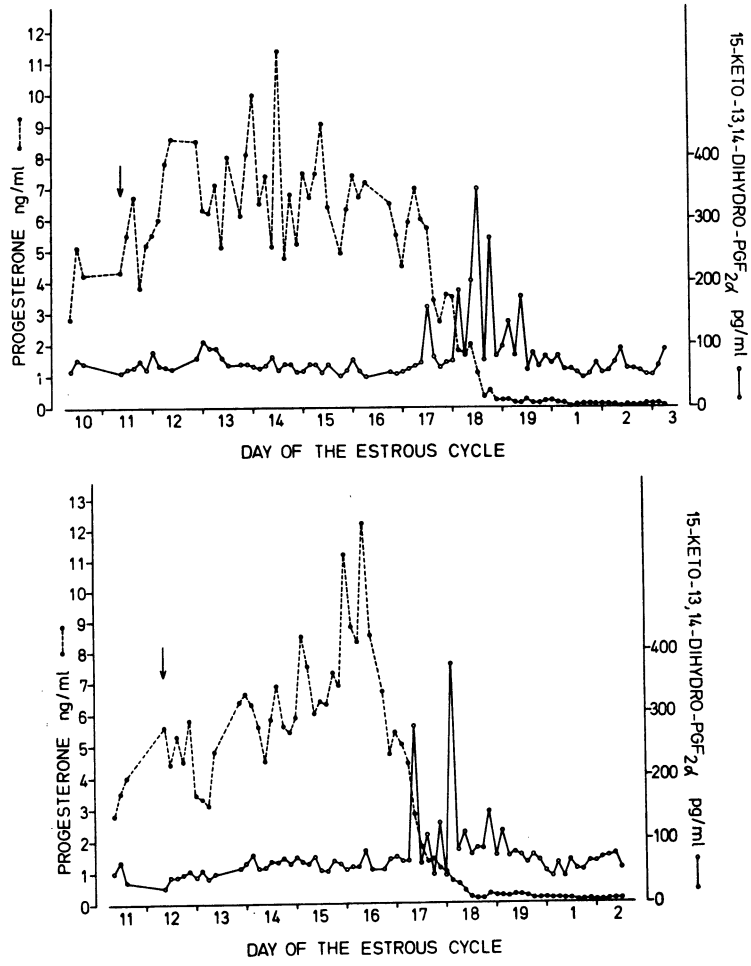

Figure 2. Peripheral plasma levels of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (o———o) and progesterone (•·····•) in cows no. 1 (upper panel) and no. 2 (lower panel) subjected to intrauterine iodine infusion on days 11 or 12 of the estrous cycle. Arrows denote time of infusion.

cycle length (*Morrow et al.* 1971, *Grunert et al.* 1973). Due to the induced delay of prostaglandin release, cycles will be prolonged if the iodine infusion is performed within less than five days before the normally expected time of prostaglandin release.

The reason for the delay in prostaglandin release is not known. *Seguin et al.* (1974) showed that intrauterine iodine infusion in cows caused a necrotizing endometritis. These authors postulated that the inflammatory response destroyed the source of the luteolysin, and that the synthesis of $PGF_{2\alpha}$ probably was

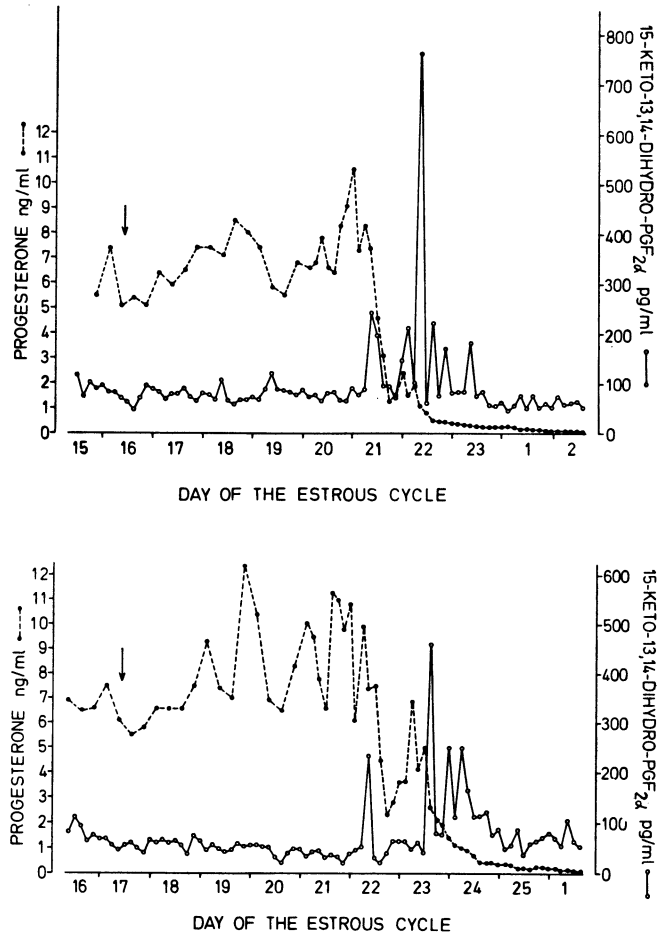

Figure 3. Peripheral plasma levels of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (o———o) and progesterone (•·····•) in cows no. 1 (upper panel) and no. 2 (lower panel) subjected to intrauterine iodine infusion on days 16 or 17 of the estrous cycle. Arrows denote time of infusion.

resumed at some point during endometrial repair. It is generally believed, that synthesized prostaglandin is not stored within the cell, but instead released immediately after synthesis. The fact that no release of $PGF_{2\alpha}$ was seen immediately after iodine infusion supports the idea that $PGF_{2\alpha}$ is not stored in the cell. Intrauterine iodine infusion apparently does not block the luteolytic action of $PGF_{2\alpha}$, since $PGF_{2\alpha}$ administered exogenously at the time of endometrial irritation on day 15 (estrus = day 0 in this experiment) shortened the time interval between infusion and occurrence of heat (*Seguin et al.*).

The physiological mechanisms responsible for the release of $PGF_{2\alpha}$ at a precise time during the bovine estrous cycle are not fully understood. After destruction of the endometrium, the nor-

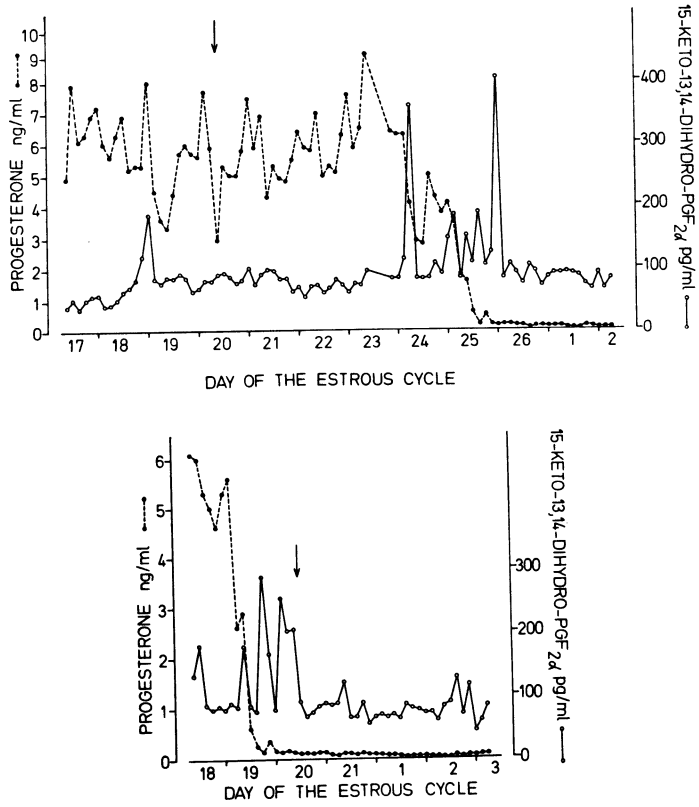

Figure 4. Peripheral plasma levels of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (o———o) and progesterone (•·····•) in cows no. 4 (upper panel) and no. 3 (lower panal) subjected to intrauterine iodine infusion on day 20 of the estrous cycle. Arrows denote time of infusion.

mal control mechanism does not seem to function. *Wlodawer et al.* (1976) have shown that the bovine endometrium contains an inhibitor of prostaglandin biosynthesis. It is possible that, during repair, a time-lag exists between the reestablishment of the prostaglandin inhibitory system as compared to the prostaglandin synthesizing system. However, further studies on the nature of the inhibitory factor are necessary for a better evaluation of its physiological significance for prostaglandin synthesis and release.

The temporal aspects of luteolysis following iodine infusion appeared to be similar to that which occurs under physiological conditions (*Kindahl et al.* 1976 a, b). There are considerable variation in the time required for luteolysis ranging from about 30 hrs. to 60 hrs. The extremes of this range are shown in Fig. 3. Both cows infused late in the cycle (days 16 and 17) began to release significant amounts of the prostaglandin metabolite at the same time after the infusion, namely 117 hrs. Yet the interval to estrus is approx. 24 hrs. longer in one cow, probably due to a prolonged interval between the first and second $PGF_{2\alpha}$ peaks. This delay allowed the corpus luteum to recover partially, as reflected by a pronounced rebound in the progesterone levels, thus prolonging the time for regression. Variations in $PGF_{2\alpha}$ release patterns during luteolysis in normal cows may well account for some of the variation observed for estrous cycle lengths.

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SAMMANFATTNING

Progesteron och 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ nivåer i den perifera blodcirkulationen hos kor efter intrauterin jodtillförsel.

Effekten av intrauterin jodtillförsel (jodsköljning) på östralcykellängden har undersökts på fyra SRB-kor. Jodinfusioner gavs tidigt, mitt i och sent i östralcykeln samt även under luteolys. Blodprov uttogs från vena jugularis en gång var 3:e timma. Blodplasmanivåerna av progesteron och 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ (huvudmetabolit till prostaglandin $F_{2\alpha}$) bestämdes för att avgöra lutealaktivitet respektive prostaglandinfrisättning. Ingen frisättning av prostaglandin erhölls i direkt anslutning till jodinfusionen. Intrauterin jodtillförsel till två kor på dag 5 av östralcykeln resulterade i prostaglandinfrisättning 54 och 69 timmar senare, vilket orsakade luteolys och brunst 5 dagar efter infusionen. Jodtillförsel på dag 11 eller 12 av östralcykeln resulterade i prostaglandinfrisättning 147 respektive 120 timmar senare, och efterföljande brunst efter en 19-dagars östralcykel. Intrauterin jodtillförsel på dag 16 och 17 av östralcykeln medförde prostaglandinfrisättning 117 timmar senare hos båda djuren. Hos den ena kon var östralcykellängden normal medan den andra hade en förlängd östralcykel. En ko infunderades på dag 20 i östralcykeln just efter det att den normala prostaglandinfrisättningen startat, och denna frisättning blockerades tillfälligt så att östralcykeln förlängdes till 26 dagar. Ytterligare en ko erhöll intrauterin jodtillförsel på 20:e dagen av östralcykeln, dock var luteolysen i det närmaste fullständig vid infusionstillfället, och någon förlängning av östralcykeln erhölls inte. En ko som infunderades på dag 5 i östral-

cykeln uppvisade normal östralcykellängd sannolikt beroende på att corpus luteum var ofullständigt utvecklad vid infusionstillfället. Från föreliggande undersökning kan det konkluderas: 1) intrauterin jodtillförsel given efter det att en progesteronproducerande corpus luteum har utvecklats resulterar i prostaglandinfrisättning och luteolys inom 3 till 6 dagar; 2) den på så sätt inducerade luteolysen observerades i samtliga fall samtidigt med prostaglandinfrisättningen, som till sin storlek och duration liknar den vid spontan luteolys.

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