EFFECT OF INTRAUTERINE IODINE INFUSION ON LUTEAL FUNCTION AND BLOOD PGF_{2a} CONCENTRATION IN CYCLING GOATS[1]

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ABSTRACT

Studies were conducted to determine the effect of iodine infusion on the luteal function of goats, as evident by blood progesterone concentration, and on plasma PGF_{2a} levels. Ten cycling mixed breed goats were synchronized for estrus by PGF_{2a} (5 mg) and given a single intrauterine iodine infusion on day 5 and on day 15 of the estrous cycle.

Iodine infusion on day 5 (group II) resulted in shorter estrous length (8.2 days) and a 7-fold increase in plasma PGF_{2a} concentration as compared to control animals (group I) given distilled water infusion. Similar infusion on day 15 (group III), on the other hand, failed to alter the estrcus cycle length but induced a moderate increase in PGF_{2a} concentration which lasted only for a brief period. The progesterone levels declined concomitantly as PGF_{2a} levels rose after iodine infusion in group II animals but failed to decline until after 24 hours in group III animals.

The studies indicate that the endometrium reacts to the chemical stimuli and releases PGF_{2a} which, in turn, alters the luteal function.

INTRODUCTION

The literature reveals that an intrauterine infusion with an irritant solution (or endotoxin) alters the length of estrous cycle. The cows given iodine solution by intrauterine infusion during the early luteal phase result in shorter estrous cycle length but a similar infusion during the late luteal phase result in longer cycle length[1,2]. Likewise, a gel-like antibiotic preparation given to cows by an intrauterine route shortens the cycle length when given during early luteal phase and prolongs the cycle length when given during the late luteal phase[3].

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The mechanism by which such infusions alter the luteal function is probably mediated by the synthesis and release of PGF_{2a} from the uterine tissues which exerts its luteolytic effect. Intrauterine iodine infusion in cows results in enhanced PGF_{2a} release[4] and lowers blood progesterone concentration after 3-4 days of treatment[5]. In contrast, in cycling goats liposaccharide endotoxin of salmonella typhimurium given into the lumen of uterine horn ipsilateral to the corpus luteum, fails to exhibit any change in PGF_{2a} or progesterone concentrations[6].

The aim of the present study was to examine the ability of iodine solution to release PGF_{2a} from the uterine tissues in goats and to assess its influence on the estrous cycle length and blood progesterone concentration.

MATERIAL AND METHODS

Animals:

Ten mature does of mixed breed (obtained from a herd maintained at the Veterinary Research Center, Moton Field) had 20-22 days of estrus cycle, when observed for standing estrus for two consecutive cycles. A vasectomized buck was allowed to run with the experimental does to facilitate detection of estrus. The experiment was conducted between the months of December and February when the does are in peak sexual activity.

Treatment Groups:

A single intramuscular injection of 5mg PGF_{2a} (lutalyse, Upjohn Company, Kalamazoo, MI) was given to all experimental does for estrus synchronization between day 6 and day 16 of estrous cycle (day 0 = estrus). The animals were randomly divided into 3 groups. Four groups I animals served as controls and were given intrauterine infusion of distilled water (20 ml) on day 5 and on day 15 of the cycle; three group II animals were given 20 ml of iodine solution containing iodine (1 gm), potassium iodide (3 gms) and water (300 ml) via intrauterine route on day 5; and three group III animal were given the same treatment as group II animals except that the infusion was given on day 15. The experiment was replicated three times in such a manner that for each subsequent replicate group I animals were assigned to group II, group II to group III, and so on, such that each set of animals was represented at least once in each treatment group.

Intrauterine Infusion:

A glass speculum with attached light was introduced into the vagina and a 17-inch long flexible plastic insemination tube was passed through the cervix into the uterus. A syringe was attached to the insemination tube via 2-inch long rubber tubing for infusion of 20 ml volume.

Blood Sample Collection:

Two separate vacutainers were used for blood collection (by a jugular venipuncture), one for plasma (PGF_{2a} assay) and the other for serum progesterone assay. For baseline data, blood samples were collected from the normally cycling animals during the pre-experimental period on day 0 (estrus), 5, 6, 7, 8, 10, 15, 16, 17, 18 and 20 for progesterone and PGF_{2a} assays. To determine the effect of infusion, blood samples were collected immediately before the infusion and then once daily for 3 consecutive days. Immediately after collection, blood samples were placed on ice and transferred to the laboratory where the plasma or serum fractions were separated. The samples were then stored in 2 ml tubes in the freezer at 20°C until assayed.

Assay of PGF_{2a}

Extraction and Column Chromatography: PGF_{2a} was first extracted and then separated by column chromatography prior to the assay as reported earlier from our laboratory[7]. Briefly, petroleum ether was added to the plasma samples and the ether phase was removed. To the ether phase, a mixture (2 ml) of ethyl acetate, isoprapanol and 2N HCL (3:3:1 v/v/v) was added, centrifuged, and 2 ml of ethyl acetate and 3 ml of water were added. The samples were mixed and centrifuged again at 4°C for 5 minutes at 1,200 x g. The organic layer was aspirated into tubes and placed in a water bath at 55°C for drying by air stream. After cooling, the tubes were stored in the freezer until column separation. Columns were made from 10-ml disposable glass pipettes and contained glass wool. A slurry, made from silicic acid, benzene and ethyl acetate was poured into the column and major fractions of prostaglandin were eluted serially with increasing concentration of methanol in a mixture of benzene and ethyl acetate. Fraction I (PGA, PGB) and fraction II (PGE) were discarded, while fraction III which contained PGF was dried by bubbling compressed air via the hypodermic needed into the tubes placed in the water bath at 55°C. The dried fraction was then dissolved in isogel Tris buffer for assay.

Assay Procedure: The assay was performed by using a commercial kit (Clinical Assays, Cambridge, MA.) which has previously been tested in our laboratory[7]. The lypholized reagents were reconstituted with gel Tris buffer and kept on ice throughout the assay procedure. Each sample including control, standard or unknown were run in duplicate. The standard curve was prepared with 6 points ranging from 9.9 pg to 2,400 pg of PGF_{2a}. For each set of determinations, 12 tubes with 6 different standard dilutions, 2 tubes with tracer only, and 2 tubes with tracer and antisera were run concurrently with the unknown samples.

Validation: Cross reactivity between antiPGF_{2a} serum at 50% inhibition of maximum binding was reported to be 28.2% with PGF_{1a}, 1.2% with 13, 14-dihydro-15-keto-prostaglandin F_{2a} and 1% with PGA, PGE_1 and PGE_2 (Clinical Assay, Cambridge, MA.). The recovery rate after extraction and chromatography was determined by adding labeled PGF_{2a} of known count to 50 ml of gel Tris buffer and 1 ml of plasma. After extraction and column chromatography, such tubes were counted and calculated for recovery rate. Mean percent recovery for 3 sets of determination (4 tubes/set) was calculated to be 61.2% (± 2.46). Precision of the assay was further ascertained by adding a known quantity of PGF_{2a} to the gel Tris buffer and assaying it along with other eluted samples. The mean percent recovery for 12 such tubes was 80.6% (± 2.73). The sensitivity of the assay was 9.9 pg/ml, which was the lowest concentration on the standard curve.

Assay of Progesterone:

The commercial kit used for this assay (Diagnostic Products Corp., Los Angles, CA.) was antibody coated, no extraction, solid phase ^{125}I radioimmunoassay designed for assaying as little as 0.05 ng/ml progesterone from serum samples. Labelled progesterone (1.0 ml) was added to tubes, in duplicate, containing 10 ul of unknown or standard. After 3 hours of incubation at the room temperature, the tubes were decanted thoroughly and were allowed to drain for 3 minutes. The tubes were counted for one minute in a gamma counter and the progesterone concentration was interpolated from the standard curve with 6 points of concentration (0.1, 0.5, 2, 10, 20, 40 ng/ml).

Validation: The assay was validated by inter-assay and intra-assay coefficient of variation. For determining the inter-assay coefficient of variation, 3 tubes in duplicate, containing a known amount of progesterone (1.76, 2.38 and 6.01 ng) were assayed along with each set of three determinations and the coefficient of variation was calculated from their count per minute (CPM). The calculated values for the three sets of determinations were 4.8%, 0.51%, 19.2% respectively, with an overall average of 7.9%. For intra-assay coefficient of variation, three known quantities of progesterone (in duplicate) (1.14, 1.98, 2.90 ng) were assayed along with each set of determinations, their coefficient of variation was 0.10, 2.10 and 2.15% respectively, with an overall mean of 1.4%.

RESULTS

On day 0 (estrus) the untreated normally cycling does (Fig 1) had relatively higher plasma PGF_{2a} concentration (mean ± SE = 355. 30 ± 21.1 pg/ml) whereas mean progesterone concentration was at the lowest level (0.5 ± 0.06 ng/ml). On day 5 (next sampling period) of estrus, PGF_{2a} levels showed a sharp decline and continued at this low level until day 17 when they started to increase, reaching a peak (464.9 ± 16.0 pg/ml) on day 20. The progesterone levels, on the other hand, showed a opposite trend, they started to increase gradually after day 0 and reached a peak on day 17 (8.85 ± 0.31 ng/ml), thereafter the values showed a sharp drop and on day 20 they reached a low level (0.91 ± 0.08 ng/ml).

A single iodine infusion on day 5 resulted in the onset of estrus within 2-4 days of treatment (table 1), their estrus cycle length varied from 7 days in 3 animals to 9 days in 5 animals (mean = 8.2 ± 0.29). In contrast, estrous cycle length after infusion on day 15 showed inconsistent results, two animals failed to show any change in estrous cycle length, 5 animals showed a prolonged length (1-3 day) and 3 animals showed a shorter estrous cycle length with an overall mean of 21.2 ± 0.4 days.

TABLE 1. THE EFFECTS OF INTRAUTERINE IODINE INFUSION ON THE ESTROUS CYCLE LENGTH OF GOATS

Day of Infusion	Group	No. of Goats	Treatment	Estrous Cycle (Days ± SE) Pre-treatment	Estrous Cycle (Days ± SE) Post-treatment	Interval from infusion to estrus (Days ± SE)
5	I	5	Distilled Water	20.7 ± 0.82	20.8 ± 0.25	5.8 ± 0.10
15	I	5	Distilled Water	20.7 ± 0.82	20.8 ± 0.25	15.8 ± 0.15
5	II	10	Iodine	20.7 ± 0.82	8.2 ± 0.29	3.2 ± 0.7
15	III	10	Iodine	20.7 ± 0.82	21.2 ± 0.49	6.5 ± 0.18

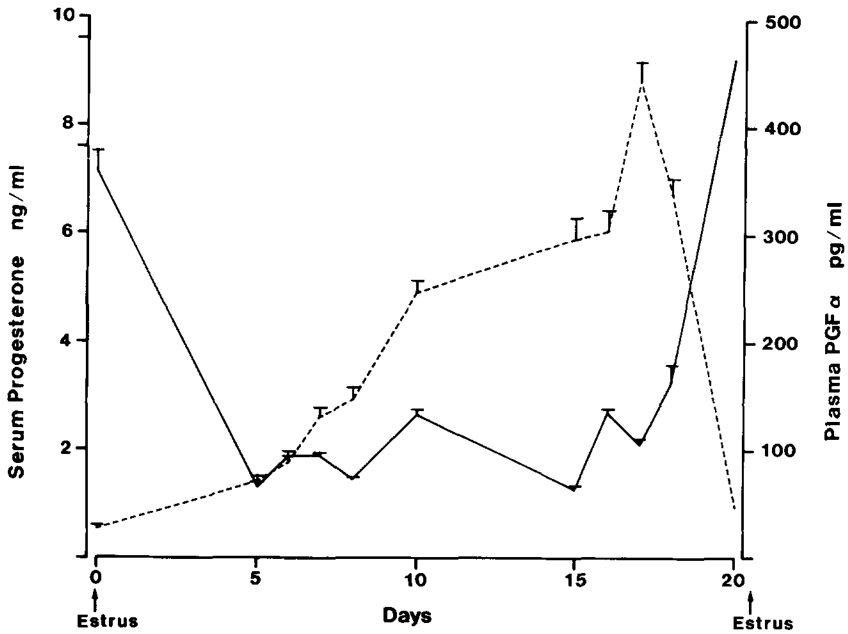

Fig. 1. Blood PGF_{2a}(solid line) and progesterone (broken line) concentration in cycling goats. Each value is a mean of 10 observations, the bars indicate standard error of means (SEM)

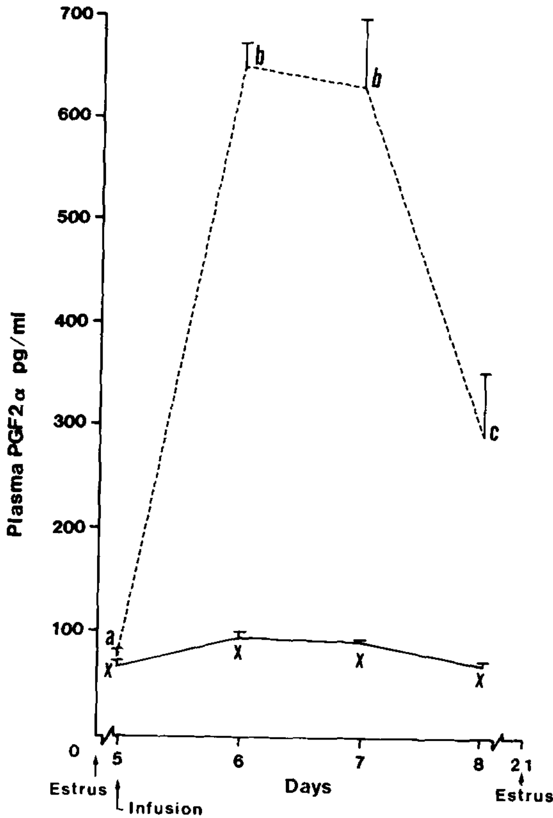

Fig. 2. Blood PGF_{2a} concentration in does after intrauterine iodine infusion on day 5 (broken line - iodine infusion, solid line - distilled water infusion). Each value is a mean of 10 observations, the standard error of means is shown by bars. Similar scripts within the same group are not significantly different ($P \leq 0.5$).

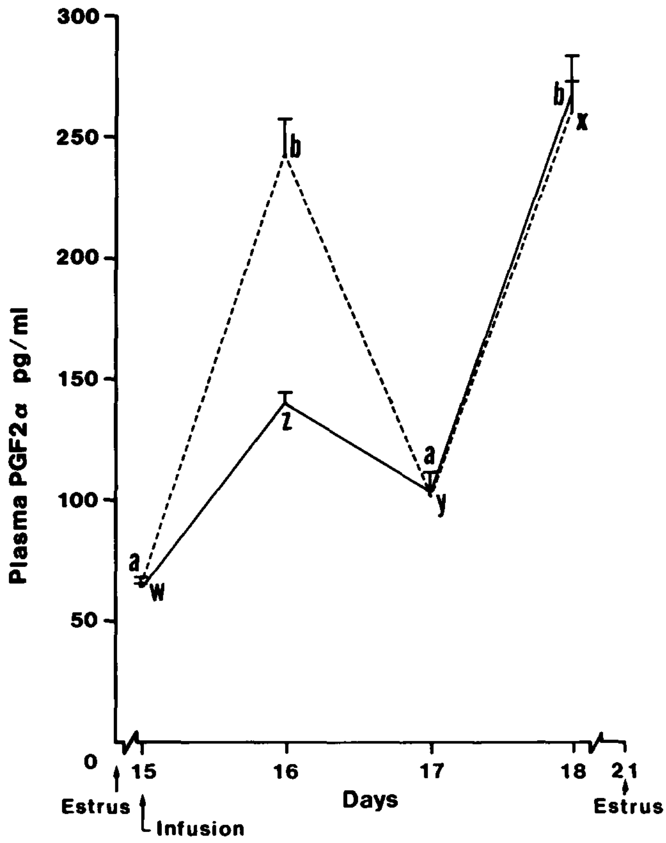

Fig. 3. Blood PGF_{2a} concentration in does after intrauterine iodine infusion on day 15. (See Fig. 2 for details).

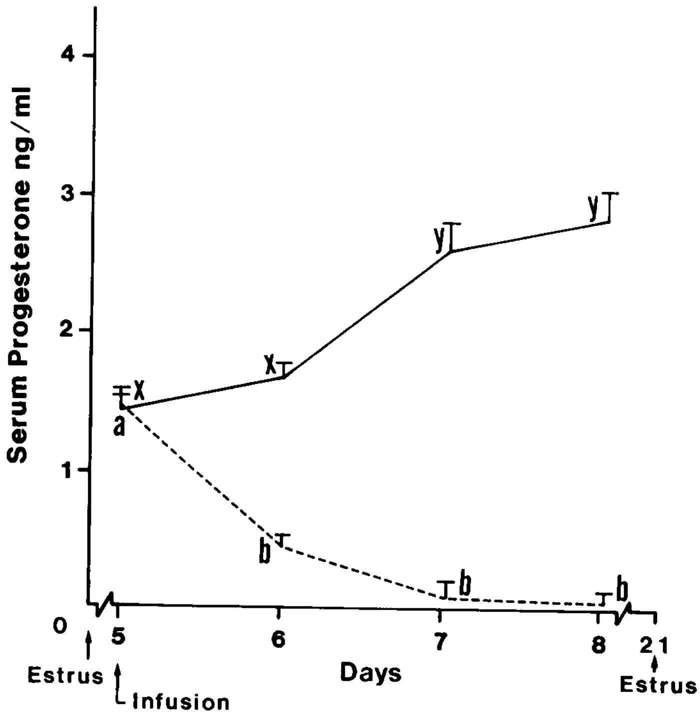

Fig. 4. Blood progesterone concentration in does after intrauterine iodine infusion on day 5. (See Fig. 2 for details).

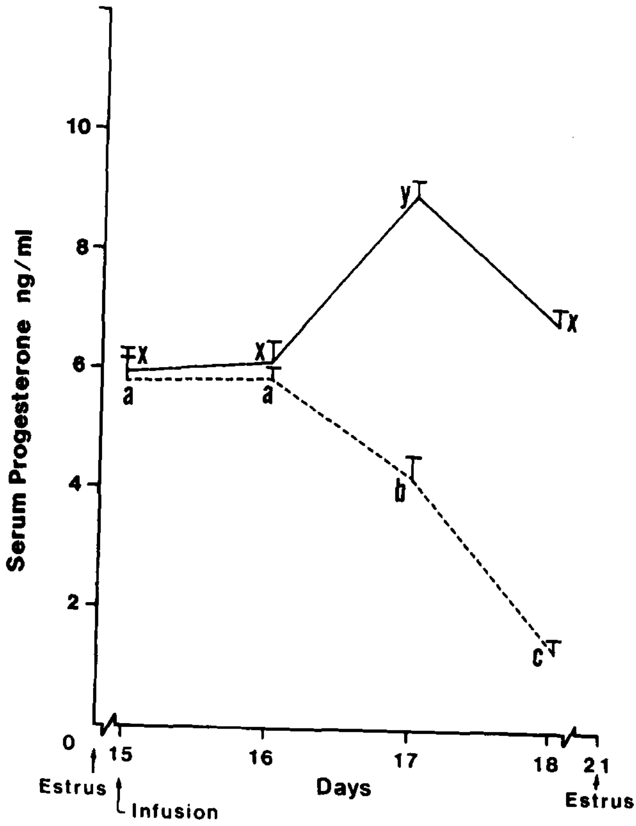

Fig. 5. Blood progesterone concentration in does after intrauterine iodine infusion on day 15. (See Fig. 2 for details).

Plasma PGF_{2a} concentration showed a 7-fold increase (650.9 pg/ml) within 24 hours of infusion on day 5 (group II) and after continuing at this level until day 7, the levels started to decline sharply (Fig. 2). However, on day 8 the values failed to reach the pre-infusion level when further sample collection was discontinued. Infusion on day 15 exhibited a relatively moderate and less pronounced increase in PGF_{2a} concentration (240.7 ± 144 pg/ml) which declined sharply thereafter.

As PGF_{2a} levels rose, progesterone levels declined concomitantly in group II animals treated on day 5 (Fig. 3) reaching a new low on day 8 (0.06 ± 0.01 ng/ml). However, in group III animals (treated on day 15), decline in progesterone concentration was not as prompt, the levels failed to show any decrease until after day 16 reaching a low level on day 18 (Fig. 4).

DISCUSSION

This experiment has demonstrated that an irritant solution placed into the uterine lumen during early luteal phase (day 5) enhances blood PGF_{2a} concentration, and alters the luteal function as noted by shorter estrous cycle length and lower serum progesterone level. The excessive release of PGF_{2a} from the uterus is probably due to the presence of large amount of arachidonic acid, a precursor of prosaglandin, present in the endometrial tissue as reported from cows[8]. Obviously PGF_{2a} is transported from the uterus to the ovary to exert its luteolytic effect. The mechanism for such transport in does may be similar to ewes as postulated earlier[9], which states that PGF_{2a} of uterine origin is transferred from the uterine vein to the ovarian artery and reaches the corpus luteum where it causes luteolysis. Whether PGF_{2a} which causes luteal regression originates solely from uterus is not clearly known. However, recent studies have shown that oxytocin secreated from the corpus luteum of cows and sheep in high quantities[10] is involved in luteal regression[11]. It (oxytocin) delays luteolysis when infused continuously during the early luteal phase probably through its inhibitory action on PGF_{2a}[12], and when injected subcutaneously, it reduces the estrous cycle length significantly in cows[13].

In spite of the uterine release of PGF_{2a} which caused luteolysis in this study, role of ovaries cannot be ruled out. Both uterine and ovarian tissues produce PGF_{2a} and other prostanoids such as the PGE and PGI_2 in humans, sheep and cows[14-16]. The goats produce PGI_2 from the luteal cells as well as from granulose cells[17]. It appears that prostaglandins of either origin is required not only for normal luteolysis but also for normal formation and development of corpus luteum[13].

Contrary to some findings in cows[2,4] this experiment failed to prolong the estrous cycle length in goats after iodine infusion during the late luteal phase (day 15). It could be attributed to the insufficient production of PGF_{2a} but less likely due to the delayed response reported elsewhere[4]. It takes approximately 5 days after intrauterine infusion during the late luteal phase for PGF_{2a} release to occur in cows[4]. However in the present study, infusion on day 15 resulted in only a moderate (259.4 pg/ml) but transitory increase within 24 hours of infusion which was probably not sufficient to cause luteolysis. In contrast, PGF_{2a} release after day 5 of infusion was intense, it reached a level (650.9 pg/ml) similar to that observed under physiological condition of luteolysis.

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