

Iodine in Milk and Serum Following Intrauterine Infusion of Lugol's Solution

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ABSTRACT

Due to their low cost, proven effectiveness, and lack of withholding time for milk and meat products, iodine solutions have been a popular choice in the treatment of dairy cow uterine abnormalities for over 50 years. This study was aimed at determining the quantity of iodine appearing in milk following the intrauterine administration of 60 mL of a 10% Lugol's solution. Two groups of 6 cycling, reproductively normal, Holstein cows under similar management and feeding programs were used. The infusions occurred in both groups on Days 1, 10, 20, 30, 40, and 50 postpartum. Control animals were infused with 60 mL physiological saline. Milk and serum iodide concentrations were measured using a double-junction reference electrode (Ag/AgCl) and an iodide/cyanide specific electrode (I/CN). Serum pH was determined using a Corning pH/ion meter (Model 150). There appeared to be no trend between days postpartum and percent of intrauterine iodine excreted via the mammary gland. The mean half-life of iodine of all cows at all infusions was 48.4 hours, with a standard deviation of 19.0 hours. No statistically significant difference was found for iodide clearance rates from the mam-

mary gland as a function of postpartum. Eighty-nine percent of all peak serum iodine concentrations occurred within 30 minutes of infusion of intrauterine Lugol's solution. Significant concentrations of milk iodide persisted in Lugol's treated cows throughout each 10-day infusion period when compared with the saline-infused group, such that no explicit recommendation for withholding due to excessive milk iodide could be made. The use of iodine-containing preparations for intrauterine infusions should be used with caution as excessive iodine content of milk could reach market.

INTRODUCTION

Iodine is a trace element in human and animal nutrition essential for the formation of thyrosine, which is subsequently responsible for the maintenance of metabolic rates. In humans, excess iodine has effects on a wide range of body systems. It increases the basal metabolic rate, enhances carbohydrate and fat metabolism, raises body temperature, and speeds the heart rate. Iodine deficiency or excess can be manifested as hypo- or hyperthyroidism, respectively.^{1,2} Thyroid hypofunction and goiter in children and hypothyroidism in the elderly has been attributed to excess iodine in the human food chain.³ Concern has arisen that high amounts

of iodine present in milk produced by some herds may actually significantly contribute to excessive human iodine intake via milk consumption.⁴

Cow's milk and blood iodine concentrations are a reflection of dietary iodine and milk production between individuals,⁵⁻⁹ and conditions such as metritis and mastitis.^{10,11} Iodine supplements in dairy feeds, including ethylenediamine dihydroiodide (EDDI), are the most common source of excessive iodine concentrations in milk.⁴ In addition to feedstuffs, iodine is commonly present in iodized salt and mineral supplements, teat dips, udder washes, and iodophor cleansers used on milk lines and bulk tanks.^{6,8,10,12-15} The use of iodine preparations for teat dips and in the cleaning of dairy equipment has previously been of concern because of the reported increase in milk iodine.¹⁶

In the face of controversy over dietary intake of iodine, uterine iodine therapy is still widely used in some areas.¹⁷ Iodine solutions have irritating properties that lead to luteolysis of corpora lutea.^{8,18,19} In addition to shortening the interestrus interval iodines create superficial endometrial necrosis with subsequent repair in repeat breeder cows.⁵ Iodine solutions are also popular because of their low cost, proven effectiveness, and lack of withholding time for both milk and meat products.

The goals of the current project were to collect information on the milk and serum iodine concentrations following intrauterine instillation of Lugol's solution, and to determine if the concentrations detected in the milk presented a significant increase in the iodine reaching the food chain. If significant increases were evident, further work would be warranted to establish a withholding period for milk products following intrauterine Lugol's therapy.⁶ The specific objectives of this project were as follows: 1) to determine if a relationship exists between the rate and/or quantity of iodine absorption from the uterus and the number of days postpartum, 2) to evaluate how this might influence future recommendations for milk withhold-

ing, and 3) to determine the concentrations of iodine in successive milkings following uterine instillation, thus permitting calculation of the percent of infusate eliminated via the milk.

MATERIALS AND METHODS

Two groups of 6 cycling Holstein cows under similar management and feeding programs were used. Each cow's entrance into the trial began within 24 hours postpartum, at which time it received its initial evaluation and infusion. Cows were randomly assigned to either the control or the Lugol's solution treatments group as each one entered the trial. No cow with a history of retained placenta, any systemic disease, or other reproductive abnormality was used in the trial.

Cows in Group I were infused with 60 mL of 10% Lugol's solution and cows in Group II (controls) were infused with 60 mL of sterile physiological saline solution. Milk and blood sampling schedules for both groups were identical. Jugular veins of all cows were catheterized within 24 hours before each 10-day infusion sequence began and were left in place for a period of 5 days to facilitate rapid and adequate blood sample collection. Because of the frequent sampling, the tail vein was considered a poor choice.

The infusions occurred on Days 1, 10, 20, 30, 40, and 50 days postpartum in both groups. Intrauterine infusions were made with the Lugol's or saline solution instilled by a 60 cc syringe attached to a 22" standard bovine insemination pipette, once the rod was passed through the internal cervical os. The sample schedule for each cow as it entered its designated group was as follows: the minimum volume of all the blood samples was 20 mL, collected in Vacutainer tubes (Vacutainer®, Monject Division of Sherwood Medical, St. Louis, Missouri, USA), permitted to clot at room temperature and centrifuged.

Blood (Serum) Sampling and Preparation

Baseline sample was collected just prior to

each treatment. Post-infusion samples were taken following each infusion according to Table 1. The serum was removed and frozen in 5-mL scintillation vials (American Scientific Products, McGaw Park, Illinois, USA) until time of sample analysis.

Milk Sampling and Preparation

Milk sample collections commenced at calving as the cows freshened. Two hundred forty mL of a composite milk sample were collected at each milking (morning and evening) from Day 1 to Day 60 postpartum. The whole milk samples were a composite sample of all 4 quarters. Milk weights were determined by direct reading of the milk column in a Surge Tru-test system (Surge, Brookville, Illinois, USA). The milk weight per milking was recorded and a sample was placed into a Whirl-pak (Nasco, Ft. Atkinson, Wisconsin, USA) bag. The whole milk samples were refrigerated immediately post-collection and frozen at -18°C until analysis. Chlorhexidine was used for post-milking teat dip throughout the study. The cattle ration was identical for both groups and contained no added iodine.

Standard Preparation

Potassium iodide (KI) standards (10^{-1}M , 10^{-2}M , 10^{-3}M , etc) were prepared by serial dilution from a $1.00 \times 10^{-1}\text{M}$ solution. Double distilled, deionized water (DDD-water) was used for diluting all standards and rinsing all equipment. Standard solutions not utilized within a 12-hour period were discarded because of the tendency of iodide to oxidize upon exposure to air.²⁰

Table 1. Post-infusion samples taken following each infusion.

5 minutes	2 hours	72 hours (3 days)
10 minutes	4 hours	84 hours
15 minutes	6 hours	96 hours (4 days)
20 minutes	8 hours	108 hours
25 minutes	10 hours	150 hours (5 days)
30 minutes	12 hours	6 days
40 minutes	24 hours	7 days
50 minutes	36 hours	9 days
1 hour	48 hours (2 days)	
1.5 hours	60 hours	

Beginning with the 10^{-7}M standard, the equilibrium potential of each standard solution was measured and recorded. The solutions of lowest concentration (10^{-6}M , 10^{-7}M) required a longer time to equilibrate despite using a magnetic bar and stir plate to maximize even dispersion of ions present. The time to equilibration was short (2-5 minutes) for the ionic concentrations in the 10^{-5}M range and greater.

Milk Sample Analysis

After all sample collection was completed, the samples were thawed (approximately 30 per run). The milk was permitted to reach room temperature and the milk iodide concentration was determined by use of a double-junction reference electrode (Ag/AgCl; No. 476067, Corning Science Products, Medfield, Massachusetts, USA) and an iodide/cyanide specific electrode (I/CN⁻; No. 476167, Corning Science Products, Medfield, Massachusetts, USA). A Corning pH meter, model 12-BRS (12 BRS Blood pH System, Corning Science Products, Medfield, Massachusetts, USA), with expanded scale, was used for all milk samples.

A 50-mL sample of milk was decanted into a graduated beaker. One-half milliliter of 2M nickel nitrate was added as an ionic strength adjuster to achieve electronic stability.²¹ A magnetic stirrer was placed in each sample, which was then placed on an automatic stirrer to assure uniform mixing as readings were taken.

Thorough mixing was essential due to the low ionic concentrations of both standards and samples. An initial reading was taken as soon as the sample equilibrated and the meter stabilized. Utilizing the standard closest to the initial sample reading (eg, a sample with an initial readout within the range of 6 would be in closest agreement with the 10^{-6}M standard solution) standard additions of 0.20 and 0.40 mL were added to the

sample. Following each addition and a subsequent period of mixing and equilibration, additional readings were recorded.

To eliminate contamination between samples, the double-junction reference electrode, the I/CN⁻ electrode, and the magnetic stirring bar were cleaned with the following procedure: soak for 10 seconds in 0.3% w/v disodium-EDTA/sodium dodecyl sulfate solution; rinse with double distilled de-ionized water; dip in a 50:50 acetone-water solution; and rinse with double distilled de-ionized water.

Serum Sample Analysis

After all serum sample collection and freezing was completed, approximately 30 samples per run were thawed and permitted to reach room temperature. The same double-junction reference electrode and iodide specific electrode were used for the serum as with the milk, but in conjunction with the Corning pH/ion meter (Model 150). The same meter could have been used for both milk and serum, but in the interim between the milk and serum analysis, the Model 12-BRS was replaced with an equivalent instrument. KI standard preparation was identical to that for milk iodide analysis.

Five mL of serum was transferred to the bottom portion of a 50 × 9 mm polystyrene Petri dish (American Scientific Products, McGaw Park, Illinois, USA). Fifty microliters of nickel nitrate was added to the serum. This permitted adequate sample size, plus sufficient surface area for placement of a magnetic stirring rod and the tips of both electrodes. Equilibration time of serum samples was much more rapid due to the more uniform nature of serum compared to milk. In place of the standard addition technique, only the initial reading of serum plus nickel nitrate was recorded. No additional benefit (precision) was achieved when the initial reading and subsequent standard additions were made in comparison with the initial electrical response. The method of cleaning the electrodes between samples was as described before.

Data Analysis

The slope achieved by the KI standards using the electrode is a reflection of the slope of the electrode (60 mV per dilution to the power of 10). This allows a standard curve to be used. Electrical response to different concentrations of iodide was linear in any segment.

With serum samples, the best-fit line (least squares linear regression) was obtained by plotting the log of the iodine concentration against the meter reading. This method, the least squares linear regression, is used to display observed numerical data. The parameters are adjusted to get an optimal data fit, where the sum of squared difference between an observed value and the value given by the model, has the least value or the best fit. With milk, the best fit was found by plotting the log of the iodine concentration against the log of the meter reading.

Calculation

Standard Curves

Corning Model 12-BRS-Least square linear regression line was plotted for log [KI] versus log of the meter reading, where [KI] was expressed as molar concentration and the meter reading was “meter” × 10^{range}.

Corning Model 150-Least squares linear regression line was plotted for log [KI] versus relative millivolts where [KI] was expressed as molarity. The molar concentration of iodine in the sample could then be determined from the meter reading of the activity of the sample.

Serum Data

[I⁻] as moles/liter was converted to mg/liter by multiplying by 126.9 (the atomic weight of iodine) × 10³ (= mg/liter). An example would be cow #10⁵, Day 1, 0 minutes – (1.11 × 10⁻⁵ moles I⁻/liter) × (126.9 g/mole) × (10³ mg/g) = 1.409 mg/liter.

Milk Data

[I⁻] as moles/liter and milk weight in pounds were used to obtain the total iodine per milking. An example would be cow #133, Day 1, PM milking – (5.75 × 10⁻⁷ moles/liter) ×

$(126.9 \text{ g/mole}) \times (12 \text{ lb. [milk weight]}) = 0.398 \times 10^{-3} \text{ g (total iodine present)}$.

Review of Sample Analysis Technique

Because milk has been shown to be a significant human dietary source of iodine, concentrations of milk iodine have been monitored for a number of years and some standard methodology has been devised. Iodine in milk is primarily found in the form of iodide, which lends itself well to determination concentrations using an iodide specific electrode.

Milk samples were permitted to thaw and 50 mL samples were pipetted for analysis. Supporting information exists to justify not including cream in the sample. Based on the data of 2 earlier investigations greater than 90% of milk iodide is found in the inorganic form of skim milk (81.7%-92.7%) and 3.5%-13.1% is protein-bound in the skim milk.^{22,23} Butterfat contains little to no iodine. The limits of precision of the iodide electrode remain accurate out to 10^{-6}M if adequate equilibration time is allowed even though the stated limit is $10^0\text{-}10^{-5}\text{M}$.²⁴

Justification for using direct readouts or serum iodide concentrations (in contrast to standard addition method employed with milk samples) was based on electrode recommendations supplied by the manufacturer.²⁵ Ion selective electrodes are based on ionic activity (potential) rather than concentration. They are made to suit selected ions and their output is measured against a suitable reference electrode.²⁵ Thus for an electrode sensitive to ion X:

$$E = C + S \times (\log A_x)$$

Where E is the indicated potential (mV) measured, C is the constant of the sensing/reference electrode pair, S is the slope of the electrode plot (60 mV for iodide), and A_x is the activity of ion x in the test solution.

By measuring the activity of solutions of known activity (KI standards, 10^{-1}M - 10^{-6}M) a straight line is constructed for the electrode. This plot permits determination of the slope and the value for the constant. Potentials of unknowns can then be measured

and their activities read from the calibration plot.²⁵

Clearance of iodide (CL_m) in units of volume/time, through the mammary gland may be estimated from serum and milk iodide concentration data as follows:

$$CL_m = X_m \div AUC$$

Where X^m is the amount of iodide eliminated in the milk over the 10-day period following infusion and AUC is the "area under the curve" of the serum iodine concentration as a function of time.

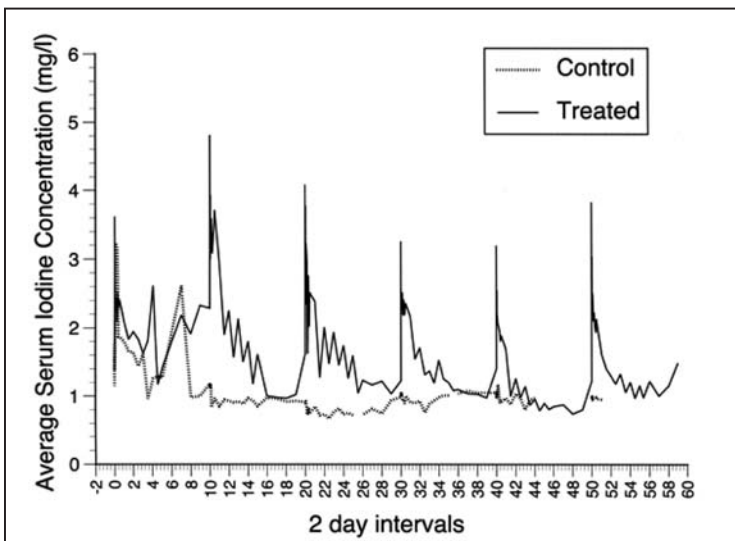
RESULTS

Serum Iodide

With only 6 exceptions, all samples taken (72 intrauterine infusions followed by 29 sampling times each) attained peak serum iodine concentrations within 30 minutes following the intrauterine instillation of Lugol's solution. Eighty-nine percent of all serum iodine maximum concentrations were reached within 20 minutes. Figure 1 represents the averages for the Lugol's solution treated cows and for the control cows in regard to absorption of iodine from the uterus and its appearance in the serum. In only one instance did one of the control cow's samples on Day 1 postpartum indicate the presence of iodine or an interfering ion. Otherwise, the serum iodine concentration of control cows remained less than or equal to 2 mg/liter.

There were only 5 instances out of the 72 infusions where the baseline concentration exceeded 2 mg iodide/liter of serum. This provided additional support that the detachable iodide concentrations were the result of the iodine infusion and not due in large part to individual variation or environmental influence. There was no recognizable trend to predict maximum iodine absorption according to day postpartum. The average time required for serum iodide concentrations to return to baseline (≤ 2 mg/liter) following uterine infusion was approximately 48 hours. Half-life determination was by Amount Remaining Excreted (ARE) calculations.

Figure 1. Averages for the Lugol's solution treated cows and for the control cows in regard to absorption of Iodine from the uterus and its appearance in the milk.



Milk Iodide

Figure 2 represents the averages for the Lugol's solution treated cows and for the control cows in regard to absorption of iodine from the uterus and its appearance in the milk.

Iodide Clearance

One-way analysis of variance failed to show any statistically significant difference between any of the postpartum iodine infusions and resultant clearance by day of infusion. The mean iodide clearance rate was 10.1 + 8.78 mL/minute.

DISCUSSION

Sample Analysis

Ion specific electrodes are in common use and their benefits stem from their relative low cost, ease of use and rapid response time.²⁵ The iodide specific electrode is suitable for both direct and known addition methods of analysis and is ideally suited for evaluation of both milk and serum samples. Following use of I¹³¹ in dairy cattle "90-100% of the I¹³¹ is in the organic form as the iodide, and the remainder is bound to proteins".²⁶ Based on this, the iodide specific electrode is able to account for the form of iodine as it is most commonly found in body

fluids²¹ Crecilius stated the level of iodide to be expected in fresh milk would be in the range of 0.5 to 3.0 mcg/mL (4-20 micromolar).²⁷ This is the range of serum iodine concentration in which the majority of cows, in both the Lugol's solution and saline treatment groups, were determined to be prior to any treatment in this investigation.

Equilibration time of samples with the electrode can be time consuming. Nickel nitrate was added to all

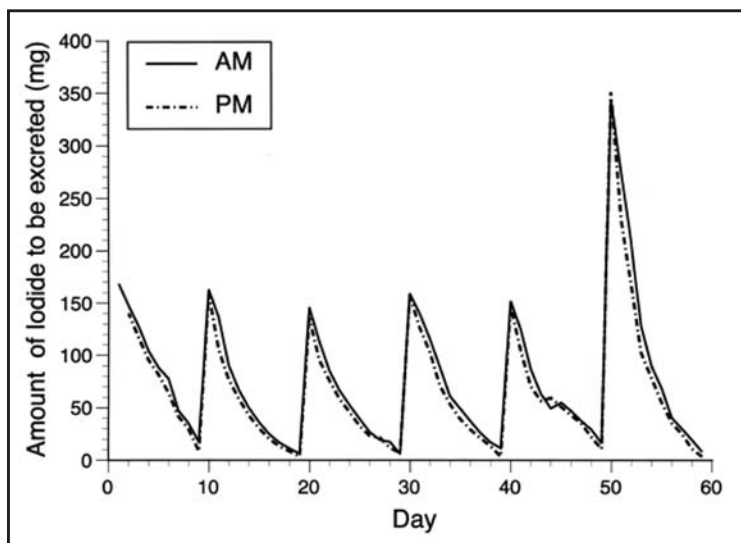
samples as an ionic strength adjuster (ISA) to lessen the equilibration time for each sample.²¹ The ISA was used with all standards and samples to maintain a high and constant background ionic strength.

Serum Iodide

Jugular catheterization aided sample collection in the immediate period after infusion. This was necessary because of the rapid uptake of iodine from the uterus. Of the 36 separate Lugol's solution infusions identified, (without regard to the elapsed time [days] following parturition) 20% of the peak serum iodide concentrations occurred within 10 minutes after infusion, 43.3% within 15 minutes, 60% within 20 minutes, and 89% of all peak concentrations were attained within 30 minutes after infusion.

Since there were no additional sources of iodine supplied in the ration, chlorhexidene was used as the post-milking teat dip, and no known outside iodine source was available other than the fed, it must be concluded that differences in baseline concentrations of iodine in various cows must be an individual variation of the animal. If environmental contamination had been a factor, more than 3 baseline samples out of a total of 72 would

Figure 2. Averages for the Lugol's solution treated cows in regard to absorption of iodine from the uterus and its appearance in the serum.



have been elevated. This lack of variation is rather remarkable considering the seemingly individual circumstances involved in the postpartum period, as well as the differences in cow body weight and milk production. The control cows again point out the small likelihood that any iodide measured came from sources other than the infusate.

Milk Iodide

There appears to be no trend between days postpartum and percent of intrauterine iodine excreted via the mammary gland. One cow eliminated 184.8 mg of iodide on Days 50 and 51 with 24% of the infused iodine appearing within the first 4 milkings. Milk production during the 2 days totaled 172 pounds, containing the equivalent of 1.07 mg iodide per pound of milk or 2,350 mcg/liter. If an infant were to consume 100 mL of this milk, it would consume 2,350 µg/liter (ie, 253 mcg) of iodine. The recommended daily intake is 35 mcg/day.²⁸ In the next 4 milkings (Days 52 and 53), 178 pounds of milk were produced containing 55.41 mg of iodide (0.31 mg per pound, or 685 µg/liter, which exceeds recommendations). This is significant considering that 100 mL of this milk, less than the volume necessary to meet nutritional needs, contains an excess of an infant's daily requirement of iodine.

Milk from Day 55 had an iodide concentration of 278 mcg/liter; Day 56, 36 mcg/liter; and Day 59, 48 mcg/liter. It appears that a relatively steady state of elimination was reached by Days 56 to 59. There is some daily fluctuation in milk production and in iodide appearance in the milk, but the iodide concentration cannot be assumed to be at a sufficiently safe level until at least 5 or 6 days following an infusion, and even then,

not with great reliability. The saving consideration is that in most situations, the cow being treated is in a larger herd. If the cow is with 9 others, the dilution factor of the other cows' milk makes it of lesser consequence and likely within acceptable limits. However, if a dairy operation had a significant problem with metritis, such that a number of the herd were treated simultaneously with Lugol's solution, the iodine eliminated in the following 4-5 milkings could be significant. Compounding of the problem is always possible in an environment in which other sources of iodine are in constant use. A dairy operation with such a history could avert most of the cumulative effects by discarding the first four milkings after intrauterine iodine treatment.

After the initial serum iodide concentration peak is reached during first 48 hours following intrauterine instillation, the daily loss (mg) is fairly constant. Since the total milligrams lost per day seems constant regardless of individual variability in milk production, the transfer of milk iodide appears to be an active phenomena. If it is an active transfer, conditions of the mammary gland, such as mastitis or udder edema, could affect collection of iodine in the milk. None of the cows in this trial were recog-

nized to be affected by such conditions during the period of sample collection.

Half-life Determinations-Milk Iodide

One-way analysis of variance failed to show differences (at 0.01 level of significance) between half-life values obtained for the different infusion periods. The mean half-life for all cows at all infusions was 48.4 hours, with a standard deviation of 19.0 hours.

Iodide Clearance Rates from the Mammary Gland

No statistically significant difference was found for iodide clearance rates from the mammary gland as a function of time postpartum. Neither iodine uptake from the uterus nor its subsequent appearance in the milk appear to be affected by the day postpartum, thus negating the anticipated effect of the enlarged postpartum uterine volume. Also, contrary to the expected outcome was the lack of remarkable changes in the iodine patterns during Days 10-20 postpartum, when the caruncles are undergoing necrosis and regression. Because none of the cows on trial had any clinically recognized uterine or mammary gland disorder at the time of the trial, we can only speculate as to the effects of metritis, mastitis, or udder edema on the uptake and clearance values of iodine.

CONCLUSION

Milk and serum iodide concentrations were monitored following intrauterine infusion of either a 10% Lugol's iodine solution or normal physiological saline in 2 groups of postpartum Holstein cows. No statistically significant difference was noted between Day postpartum for either the rate or quantity of iodine uptake. Eighty-nine percent of all peak serum iodine concentrations occurred within 30 minutes of intrauterine Lugol's solution infusion. The half-life of Lugol's iodine solution administered by the intrauterine route was 48.4 hours.

Significant concentrations of milk iodide persisted in Lugol's treated cows throughout each 10-day infusion period when compared with the saline infused group, such that

no explicit recommendation for withholding due to excessive milk iodide could be made. The greatest milk iodide elimination occurred within 5 days post-infusion. Dilution of milk from iodine treated cows by other non-treated cows on a dairy farm would lessen the impact of elevated milk iodide concentrations. These high milk iodide concentrations could significantly affect bulk milk iodide levels if more than 10% of a herd were treated at one time, if a dairy herd were small (lessening the dilution effect afforded by a large herd), or if the herds' baseline milk iodide concentration was already elevated because of other iodine sources, such as feed, sanitizing solutions, teat dips, and udder washes. No differences were noted for iodide clearance in the milk as a function of time postpartum. The use of iodine containing preparations for intrauterine preparations for intrauterine infusions should be used with caution due to their possible effect on the iodine content of milk reaching the market.

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