Target Animal Safety Study of the Use of DIB ® Intravaginal Devices (Syntex, Argentina) Containing 0.5, 1.0 or 1.38 g of Progesterone

Professionals responsible for the study:

- Dr. Leonel Alisio, Catholic University of Cordoba.
- Dr. Mariano Avilés, Catholic University of Cordoba.
- Dr. Dolores Moreno, Catholic University of Cordoba.
- Dr. Lucas Cutaia, Syntex SA.

Introduction

- This study was conducted according to the recommendations included in Guidelines VICH GL 43 (Target Animal Safety)-Pharmaceuticals dated July 2008.
- Data from safety studies including target animals (TA) are required for veterinary product registration. The international harmonization of standards for essential TA studies will be useful to adapt the data, and significantly reduce the need to perform separate studies for regulating bodies in different countries.
- Appropriate international provisions should reduce the cost involved in both research and development, and so avoid repetition of similar studied in each region.
- The VICH TAS has been designed as a harmonized provision to help conduct TAS studies to be mutually accepted by the relevant official regulating bodies.
- The use of the VICH guidelines to support product registration for local distribution is highly advisable.

Date of the study

- May 2014

Place:

- Santa Julia Zootechnical Facility, Malvinas Argentinas, Cordoba, Argentina.

Study product:

- DIB; silicone bovine intravaginal device containing 1.0 g of Progesterone. (Syntex SA, Argentina).
- DIB 0.5; silicone bovine intravaginal device containing 0.5 g of Progesterone. (Syntex SA, Argentina).
- DIB 1.38; silicone bovine intravaginal device containing 1.38 g of Progesterone. (Syntex SA, Argentina).

Target species:

Bovine female

Objective

The aim of this TAS study is to provide information about the safety of three different types of intravaginal devices with progesterone (P4) used with the target species, the bovine female. The devices are DIB (1.0 g of Progesterone), DIB 0.5 (0.5 g of Progesterone) and DIB 1.38 (1.38 g of Progesterone). The three products are manufactured by Syntex SA, Argentina.

Materials and Methods

Animals

The study included 32 San Ignacio adult bovine females (Tuli x Hereford) born between August 2009 and December 2010, i.e. about 4 and 5 years old. (Photo 1). After weaning and with an average BCS (body condition score) at treatment initiation of 3.4±0.4 (score 1-5) the cows were fed a controlled diet (30 kg of corn silage, 3 kg of ground corn, 0.5 kg of soybean expeller, per animal per day).

Photo 1: Animals included in this study (n=32).

All the cows had a tag with a number for identification (Photo 2).

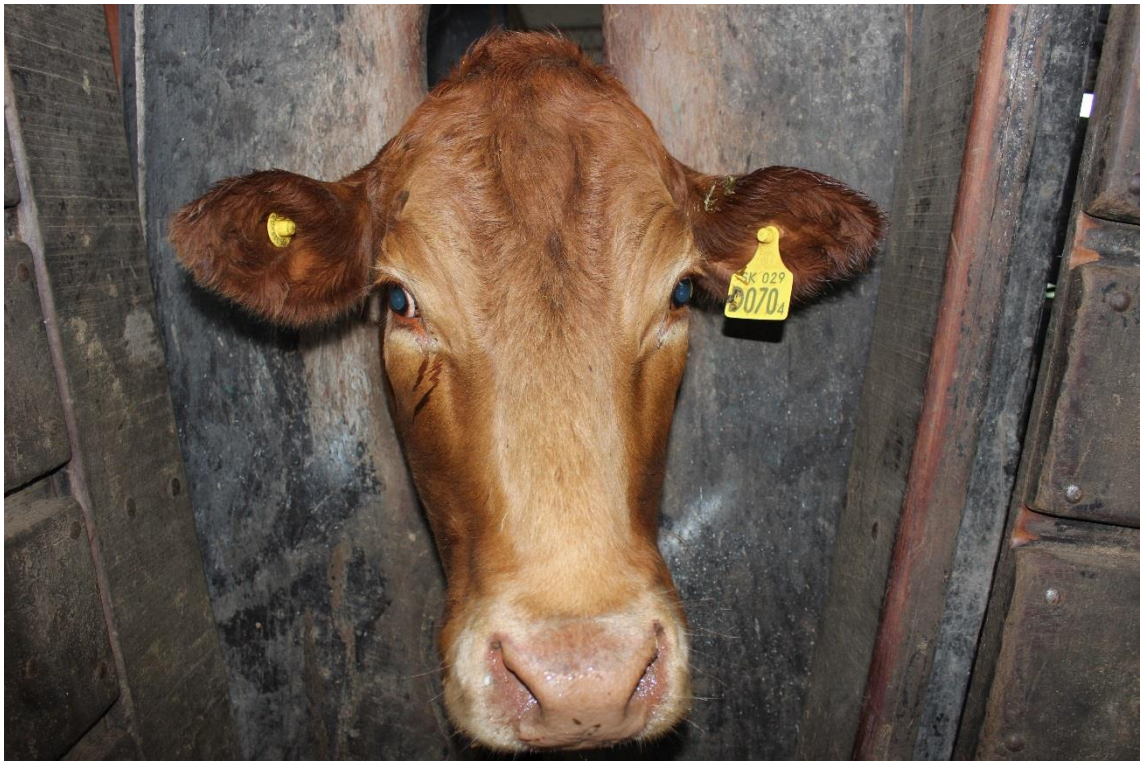


Photo 2: Animal with the tag for identification, tag n° D 070.

Treatment groups

The cows were randomized to four different treatment groups considering their BCS, weight and age; one group did not receive any treatment; the DIB Group had a silicone intravaginal device implanted containing 1.0 g of Progesterone for a period of 9 days; the DIB 0.5 Group had a silicone intravaginal device implanted containing 0.5 g of Progesterone for a period of 9 days, and DIB 1.38 Group had a silicone intravaginal device implanted containing 1.38 g of Progesterone for a period of 9 days (Table 1).

Table 1: Animals randomized to 4 treatment arms, Control Group, DIB 0.5 g Group, DIB 1.0 g Group and DIB 1.38 g Group (with a tag for identification).

CONTROL (n=8)	DIB 0.5 g (n=8)	DIB 1.0 g (n=8)	DIB 1.38 g (n=8)
318	8	10	16
2860	22	60	70
5182	114	144	164
7424	214	516	202
7425	734	742	616
7453	748	852	824
9722	846	864	844
10846	5177	2862	986

Study Products:

Photo 3: Devices used in the DIB 0.5 g Group. (Batch n° DUC 993 08/13, expiration date 08/15).

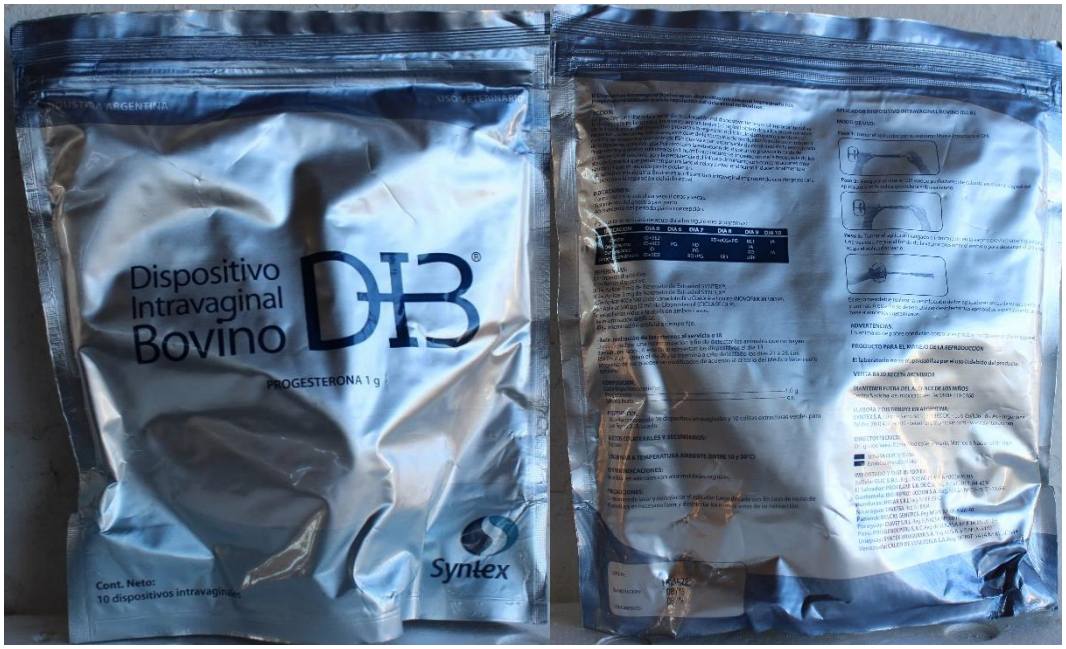


Photo 4: Devices used in the DIB 1.0 g Group. (Batch n° IND 1522 08/13, expiration date 08/16).

Photo 5: Devices used in the DIB 1.38 g Group. (Batch n° 149/ DP, 10/13, expiration date 10/15).

The devices were inserted deep in the vagina by an ad-hoc designed applicator marketed together with the product. Before inserting the device, the perivulvar area was washed with tap water and dried with paper towels. Both the devices and the applicators were disinfected with a 0.5% quaternary ammonium solution before insertion (Photos 6 and 7). On Day 9 all the devices were removed manually by pulling from the device tail.

Photo 6: The applicators are disinfected with quaternary ammonium.

Photo 7: The devices are attached to the applicators.

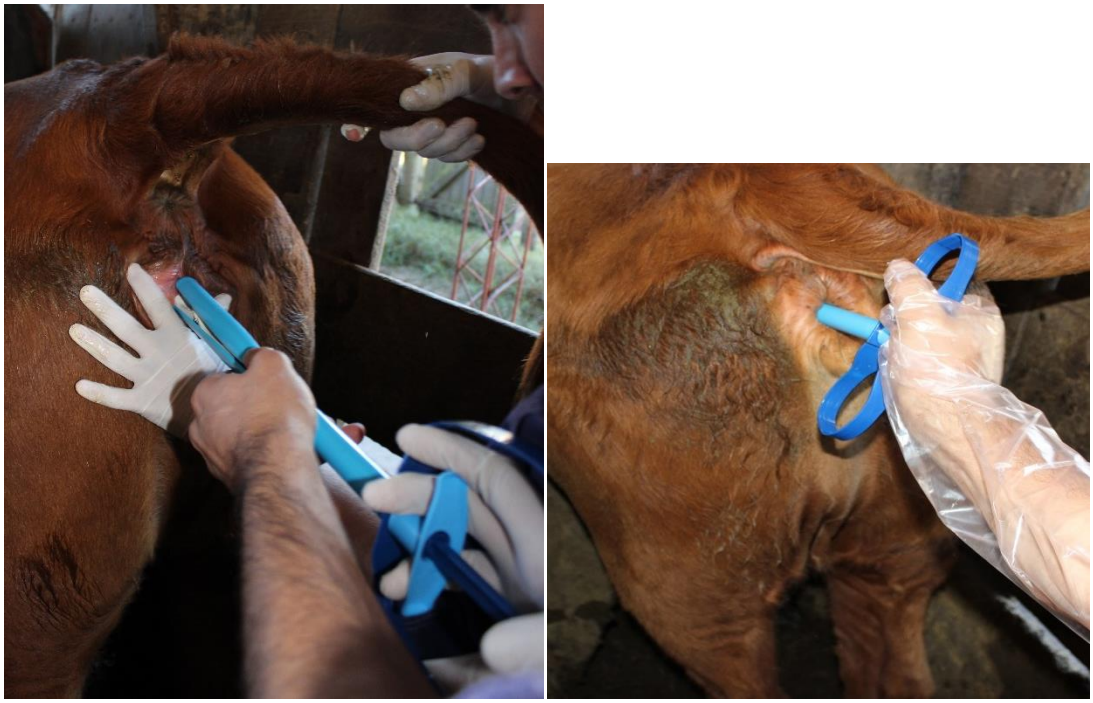


Photo 8: The device is inserted in the vagina.

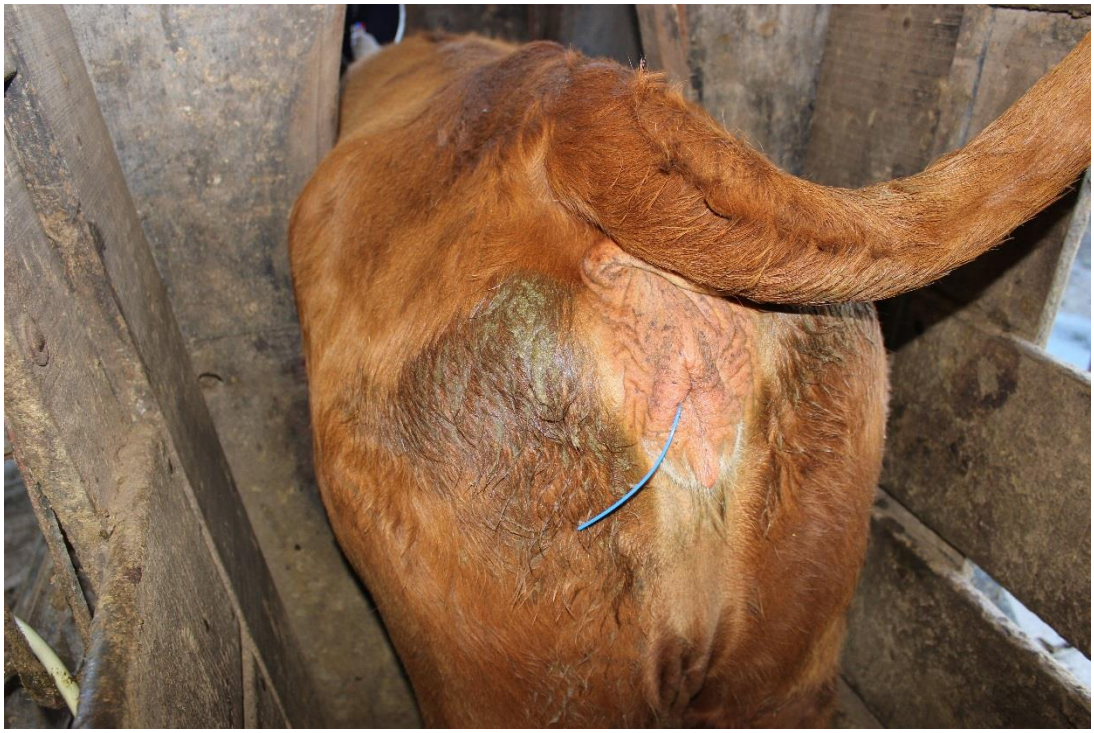


Photo 9: Animal with the DIB 1.0 g device inserted in the vagina.

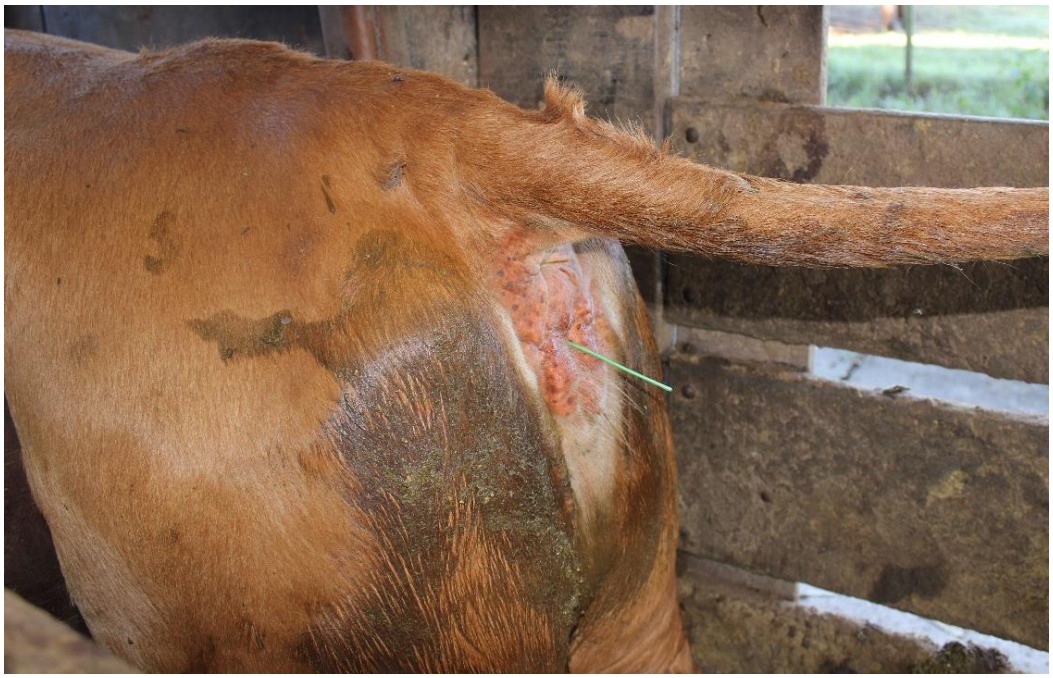


Photo 10: Animal with the DIB 1.38 g device inserted in the vagina.

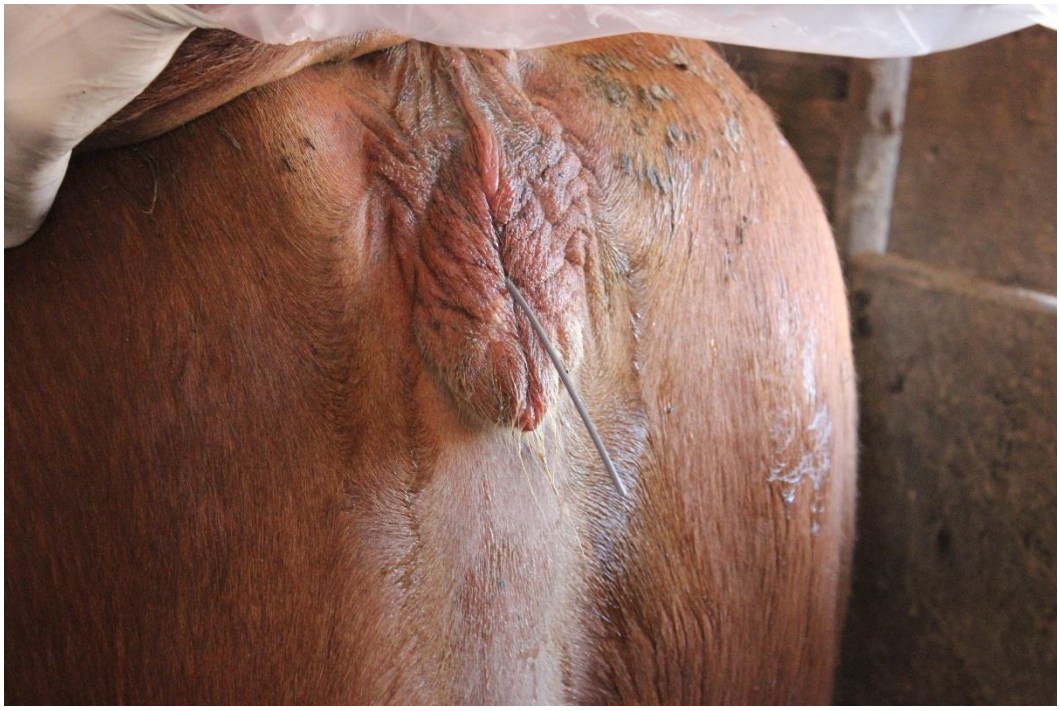


Photo 11: Animal with the DIB 0.5 device inserted in the vagina.

Laboratory tests

Seven days (Day -7) before inserting the devices, blood samples were obtained by puncturing the jugular vein (Photo 12, Annex 1: Sampling records) for a CBC (complete blood count), and to measure GOT (glutamic oxaloacetic transaminase), GPT (glutamic pyruvic transaminase) and CPK (creatine phosphokinase, as well as urea, creatinine, total proteins and fibrinogen.

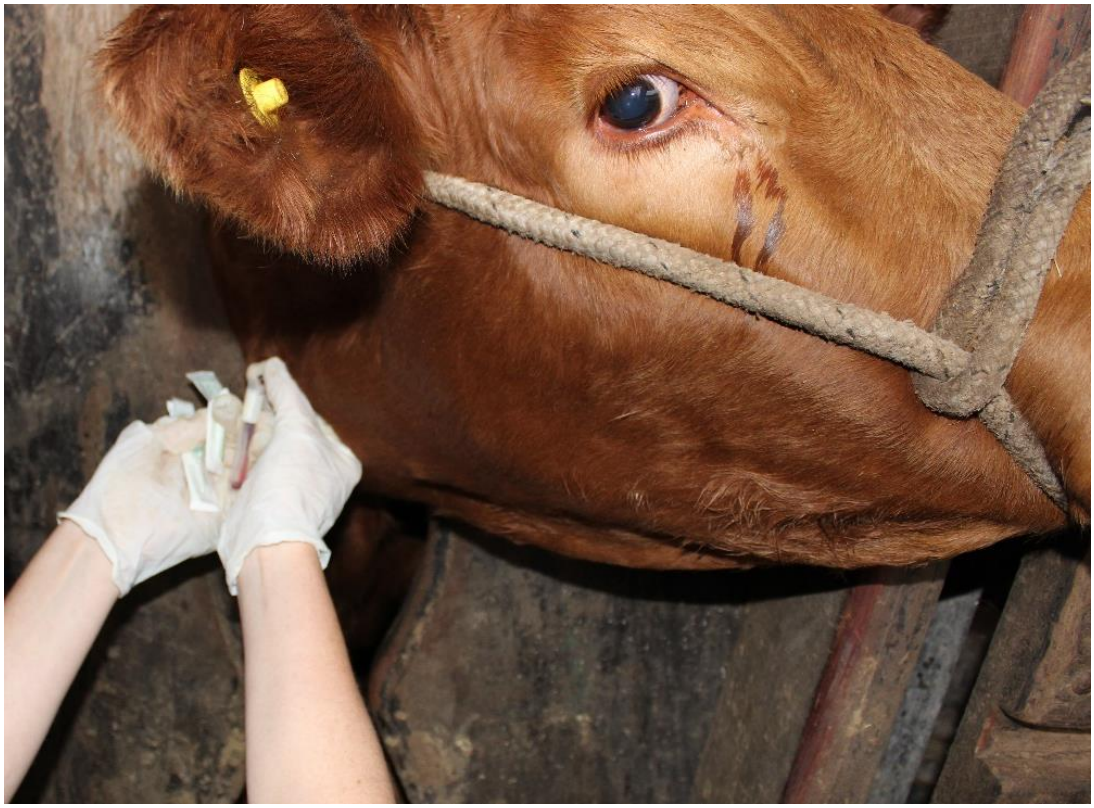


Photo 12: Blood sampling by puncturing the jugular vein.

Then, samples were obtained on Day 0, one sample immediately after removing the devices (Day 9). The last blood sample was obtained for the same measurements 7 days after removing the devices (Day 16). The samples were kept in three different tubes for the CBC (tubes with a red cap with EDTA anticoagulant), for blood chemistry panel (tubes with a green cap without anticoagulant for clot formation and serum use), and for fibrinogen (tubes with a blue cap with sodium citrate which were immediately centrifuged so plasma was separated and transferred to another tube). All the samples were then frozen (Photo 13), and processed at the laboratory of the School of Veterinary Sciences of the University of Cordoba.

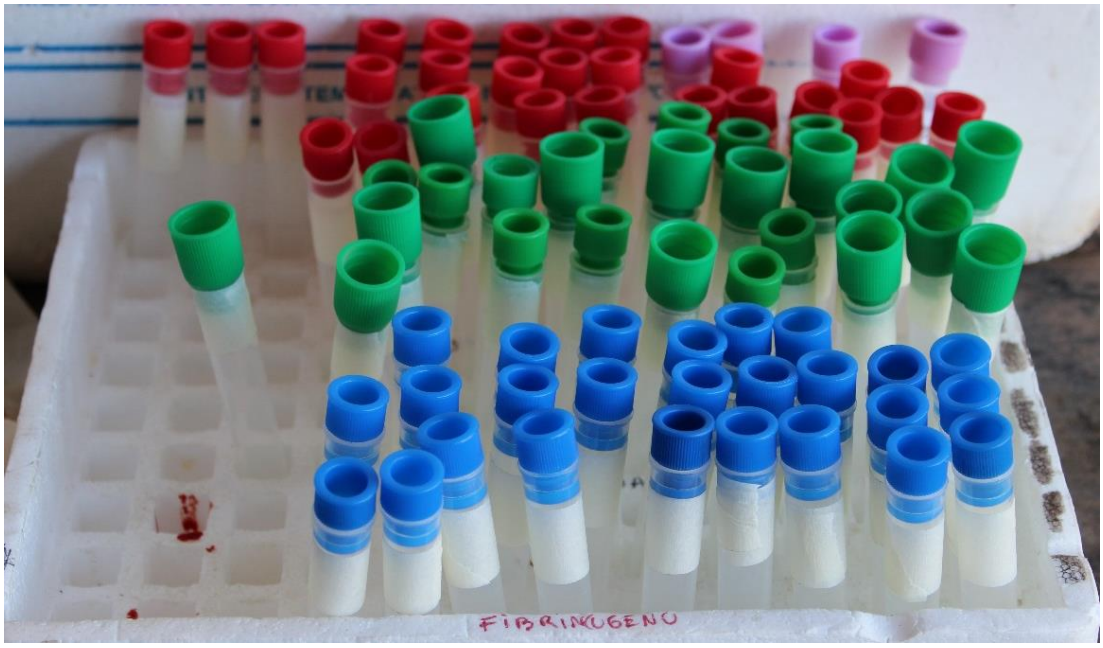


Photo 13: Tubes for blood collection for CBC (red caps), for blood chemistry panel (green caps) and for fibrinogen (blue caps). The tubes with a pink cap were taken to the chute as back up.

General clinical observation

The animals were studied from Day -7 until Day 16 considering a 9-day treatment period (Annex 2, Daily Records). General status, food and water intake were recorded during this period. The animals were classified daily as healthy or unhealthy.

Clinical examination

Apart from blood sampling, a clinical evaluation was performed before inserting the devices (Day -7 and 0). Two other evaluations were performed after removing the devices (Day 9 and Day 16). All the results were recorded in individual spreadsheets. (Spreadsheet 1, Annex 3 Individual spreadsheets).

Spreadsheet 1: Individual Spreadsheet used to record cow characteristics and parameters.

COW NUMBER		**PLACE OF WORK:**	
GENDER			
BREED			
AGE		**HAIR**	
MARKS and/or TRAITS		**TAGS or TATOOES**	
BIOTYPE		**BODY CONDITION SCORE**	
TEMPERAMENT			

WEIGHT				
TIME				
DATE	**TUES 6 MAY 14**	**TUES 13 MAY 14**	**THURS 21 MAY 14**	**THURS 28 MAY 14**
TREATMENT DAY	**DAY -7**	**DAY 0**	**DAY 9**	**DAY 16**
RECTAL TEMPERATURE (37.7 to 39 average 38.5°)				
FECES				
RESPIRATORY SYSTEM				
RESPIRATORY RATE (10 to 30 XMIN average 23)				
CARDIOVASCULAR SYSTEM				
COCCYGEAL PULSE (90XMIN)				
HEART RATE (40 to 80 average 60XMIN)				
REPRODUCTIVE SYSTEM				
VULVAR EXAMINATION				
VAGINAL EXAMINATION				
PALPATION AND US				
UTERUS				
RIGHT OVARY				
LEFT OVARY				

The following characteristics were assessed:

Weight:

Each animal was weighed in the chute using digital scales. (Photo 14, Annex 4, Weight records).

Photo 14: Digital scales used for weighing the cows.

Hair:

The color and characteristics of each animal´s hair were recorded.

Body condition score:

The body condition score (BCS) of each animal was obtained by direct observation. (Score 1-5).

Temperament:

Animals were classified as sanguineous or lymphatic.

Thermometry:

The rectal temperature was measured in degrees Celsius (Centigrade) using a digital thermometer: normal range 37.7ºC to 39.0ºC with an average of 38.5ºC (Photo 15). All the ranges are based on the Veterinary Manual published by Merck & CO., Inc. Rahway, NJ, USA; 1988.

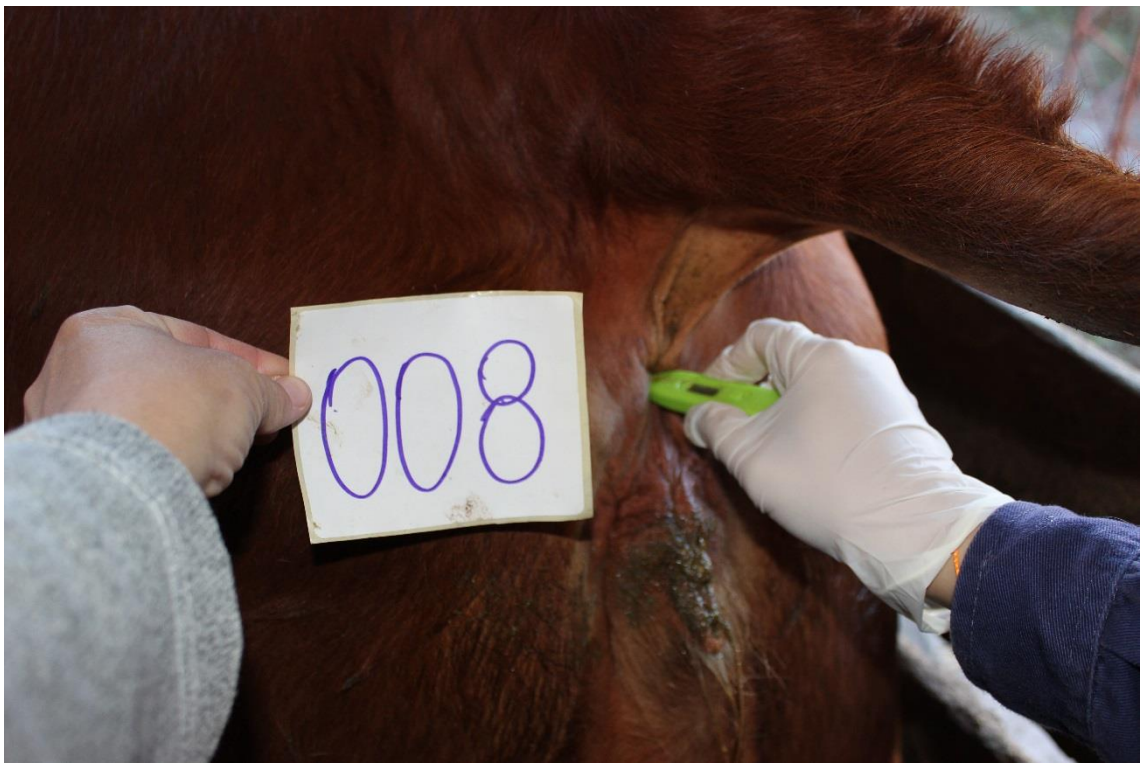


Photo 15: Rectal temperature measurement using a digital thermometer.

Pulse and heart rate:

Once the animal was quiet and immobilized in the chute with a headgate, the pulse was checked in the medial coccygeal artery located in the caudal region in the lower canal of the coccygeal vertebral bodies. An increase of over 90 beats per minute in adult bovine cattle is suggestive of a circulation disorder. This arterial pulse is determined by the heart rate, the ventricular systolic volume, arterial elasticity and peripheral resistance. The semiology of pulse assessment includes evaluating the function of the heart and the cardiovascular system. Moreover, the heart rate (i.e. the number of beats per minute including systole and diastole) was obtained using a stethoscope. The sites for cardiac auscultation are identified by drawing an 90° angle on the left side at the level of the elbow between the third and fifth intercostals spaces since the first and second intercostals spaces are covered by the scapula. In the sixth intercostal space are the tip of the heart, the lower insertion of the diaphragm and the reticular groove.

The heart rate in adult bovine cattle ranges between 40 and 80 bpm, with an average of 60 bpm.

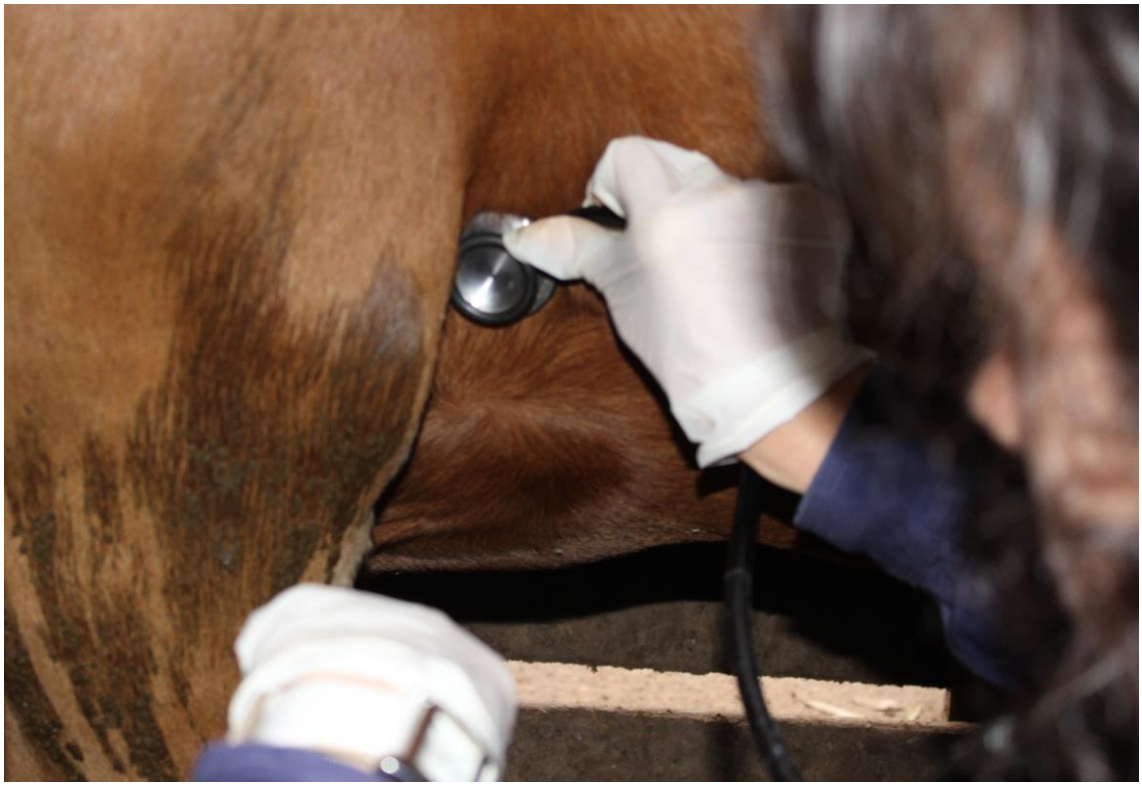


Photo 16: Heart rate in cows.

Respiratory Rate:

The respiratory rate was obtained. The respiratory rate shows the number of respiratory movements per minute. In complete cycles (inspiration/expiration), a stethoscope is used for auscultation in the pulmonary region between the sixth and seventh intercostal spaces (Photo 17). The respiratory rate in healthy adult bovine cattle ranges between 10 and 30 respiratory movements per minute, on average 23.

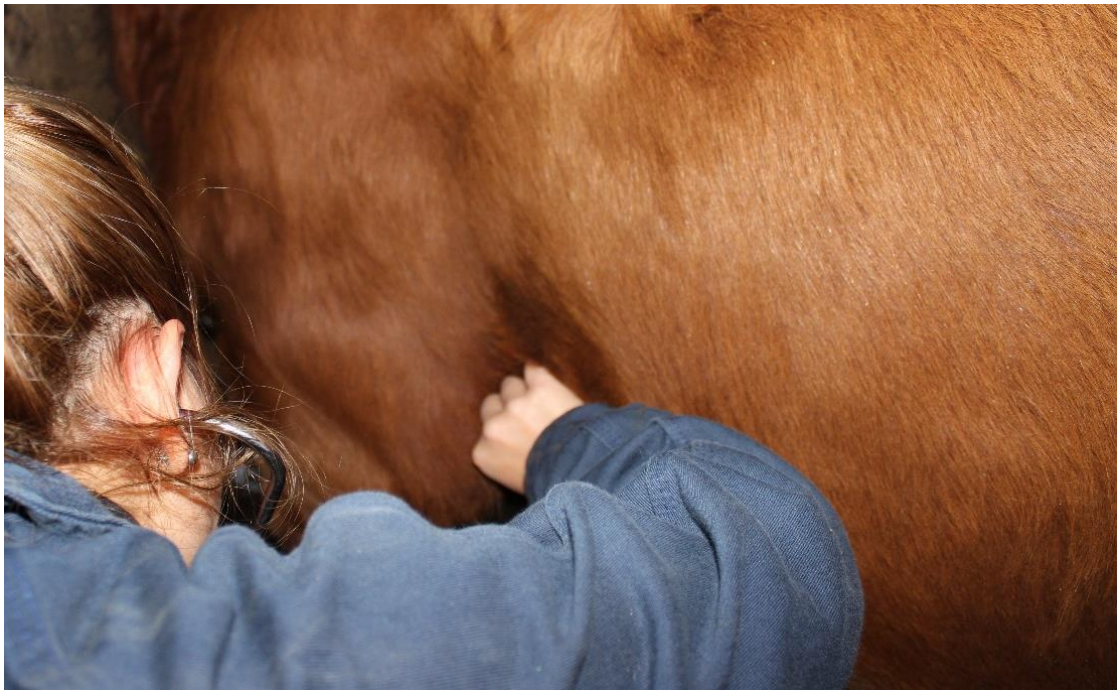
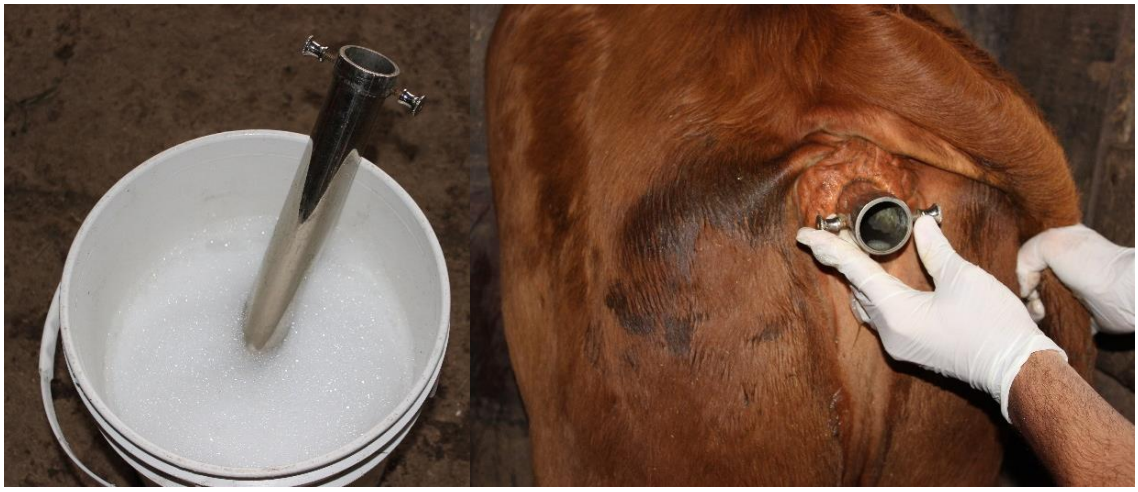


Photo 17: Measuring respiratory rate in cows.

Vaginoscopy:

A vaginoscopy was performed to visualize the vaginal fundus (ostium uteri/opening of the uterus). Photographs were taken of each vaginoscopy, and the animals were carefully identified (Photos 18 and 19).



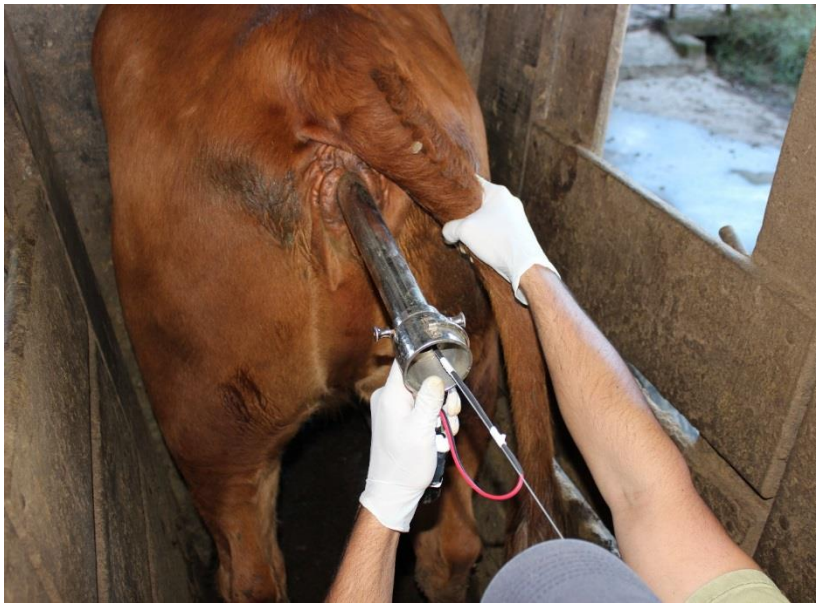


Photo 18: Insertion of the light source vaginoscope to visualize the vaginal fundus and cervix.

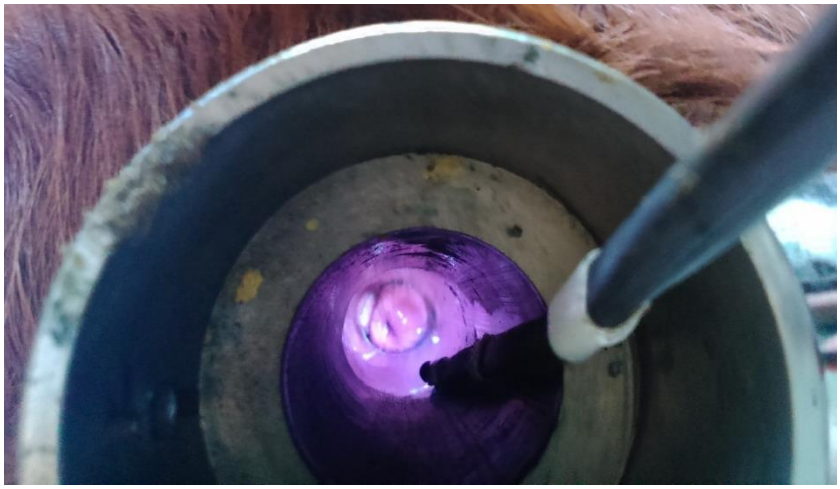


Photo 19: Visualization of the cervix with the vaginoscope

Vulva, Vagina, Uterus and Ovaries:

On examination the vulva looked unremarkable. Photographs were taken of the vulva and vagina. (Photo 20).

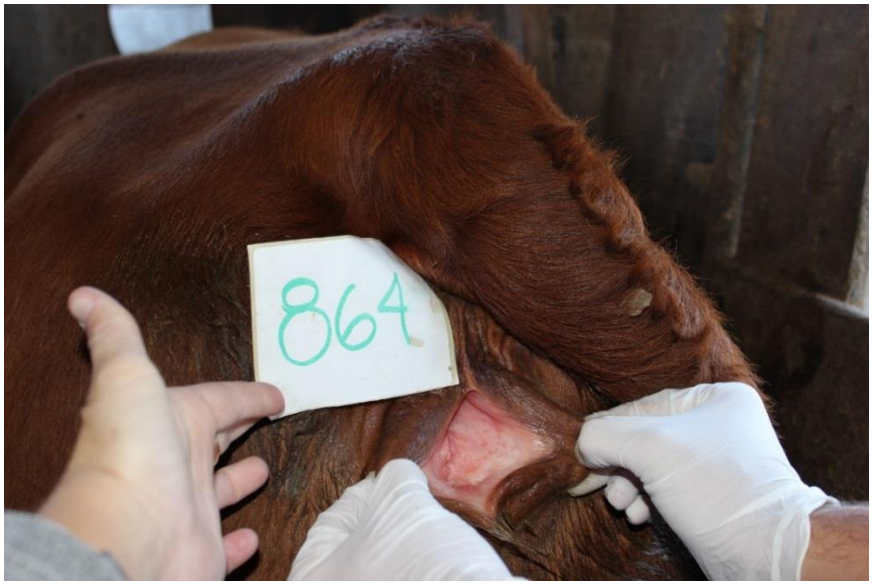


Photo 20: Opening the vulva for examination

Transrectal palpation and an ultrasound of both the uterus and ovaries of each animal were performed to assess tone, size, contents, ovarian structures (follicles, Corpus luteum) and detect any potential disorder (Photo 21). The data were plotted in an US spreadsheet. (Annex 5: Ovarian US spreadsheets).

Photo 21: Facilities (chute and headgate) where the parameters, blood samples, vaginoscopies and ultrasound (with the echograph) were obtained.

All the information was included in individual spreadsheets specially designed for data collection (Annexes 1, 2, 3 and 4). All the interventions were recorded in the lab logbook accompanied by digital records.

Statistical Analysis:

The descriptive statistics of each variable for each treatment group were estimated, and plotted, if applicable. For the quantitative variables, useful descriptive statistics will include the number of animals in each treatment arm, median and average values, standard deviation, maximum and minimum values, and the percentage of cases with values outside the reference

range. For some quantitative variables, the categorization of animals with different ranges was used to identify patterns. ANOVA or the contingency tables were used for comparison depending on the variable under study.

Results

On visual examination, the animals did not evidence either qualitative or quantitative changes. None of the animals exhibited signs or symptoms of disease during the trial. All the animals were confined in the same paddock and were fed the same ration. Annex 2 includes the daily observation spreadsheets.

Animal Temperament

Most of the animals exhibited a lymphatic temperament; only 6 of the 32 animals had a sanguineous temperament. The table below shows animal distribution in Groups (Table 2).

Table 2: Animal temperament and distribution in the treatment arms

	Control group	**DIB 0.5 g Group**	**DIB 1.0 g Group**	**DIB 1.38 g Group**
n	**8**	**8**	**8**	**8**
Sanguineous	**1**	**3**	**1**	**1**
Lymphatic	**7**	**5**	**7**	**7**

Body Condition Score

All the animals had a satisfactory body condition score at treatment initiation (Day -7), and remained under the same nutrition conditions until the end of the study on Day 16. Therefore, no variations were observed in body condition throughout the study (Table 3).

Table 3: Average body condition of the animals in each group.

	Control Group	**DIB 0.5 g Group**	**DIB 1.0 g Group**	**DIB 1.38 g Group**
n	**8**	**8**	**8**	**8**
Body condition (Day -7 to Day Día 16)	**3.2 ± 0.3**	**3.6 ± 0.5**	**3.3 ± 0.3**	**3.5 ± 0.4**

Animal Weight

No weight variations or differences were observed during the 4 days among the treatment groups. (Table 4)

Table 4: Average weight of the animals per Group during the treatment days under study.

	Control Group	**DIB 0.5 g Group**	**DIB 1.0 g Group**	**DIB 1.38 g Group**	**P**
Day -7	**458.9 ± 16.5**	**460.8 ± 33.1**	**415.6 ± 1.,7**	**441,3 ± 12.4**	**0.4**

Day 0	**454.9 ± 15.6**	**456.0 ± 34.1**	**413.8 ± 20.1**	**434,1 ± 12.7**	**0.5**
Day 9	**462.3 ± 12.1**	**464.6 ± 35.3**	**415.9 ± 18.8**	**441,6 ± 13.2**	**0.4**
Day 16	**470.6 ± 15.4**	**472.4 ± 31.4**	**431.1 ± 18.0**	**453.8 ± 14.2**	**0.5**
p	**0.9**	**0.9**	**0.9**	**0.8**	

Thermometry:

The rectal temperature was recorded during the 4 days of the study, and no significant changes or differences were observed among the study groups (Table 5). Some cows exhibited slightly high temperatures, according to their sanguineous temperament.

Table 5: Average rectal temperature per Group during the study period.

	Control Group	**DIB 0.5 g Group**	**DIB 1.0 g Group**	**DIB 1.38 g Group**	**P**
Day -7	**38.8 ± 0.2**	**38.7 ± 0.2**	**38.7 ± 0.2**	**39.5 ± 0.4**	**0.06**
Day 0	**39.0 ± 0.1**	**39.2 ± 0.2**	**39.0 ± 0.1**	**39.1 ± 0.1**	**0.6**
Day 9	**38.7 ± 0.1**	**39.1 ± 0.3**	**38.8 ± 0.2**	**39.0 ± 0.1**	**0.4**
Day 16	**38.8 ± 0.2**	**38.6 ± 0.3**	**38.9 ± 0.1**	**38.8 ± 0.2**	**0.8**
p	**0.7**	**0.3**	**0.6**	**0.09**	

Coccygeal pulse:

The coccygeal pulse of the animals was obtained during the 4-day study period, and no significant changes or differences were observed during the trial among the study groups (Table 6). The pulse was regular in terms of rate, amplitude and strength, and within the normal range for all the animals (50 to 80 per minute); however the recordings were close to the upper limits of normal since typically cows get excited when they move up a chute due to all the controls and maneuvers they are exposed to. This increasing pulse variation is the physiological response to an increased release by the adrenal medulla and activity of the sympathetic component of the autonomous nervous system.

Table 6: Average coccygeal pulse per Group during the treatment days under study.

	Control Group	**DIB 0.5 g Group**	**DIB 1.0 g Group**	**DIB 1.38 g Group**	**P**
Day -7	**80.0 ± 0.9**	**77.0 ± 2.9**	**76.3 ± 2.6**	**76.3 ± 4.5**	**0.8**
Day 0	**80.0 ± 0.0**	**80.5 ± 1.1**	**76.9 ± 2.1**	**79.5 ± 1.8**	**0.3**
Day 9	**78.8 ± 1.3**	**76.9 ± 1.9**	**78.5 ±1.6**	**81.3 ±0.8**	**0.2**
Day 16	**79.4 ± 0.6**	**75.6 ± 2.2**	**78.1 ± 1.3**	**80.6 ± 1.8**	**0,2**
p	**0.7**	**0.4**	**0.8**	**0.5**	

Heart Rate:

The heart rate of the animals was recorded during the 4-day study period; no significant changes were observed during the trial; no differences were detected among the treatment groups (Table 7). The normal heart rate in adult bovine cattle ranges between 40 and 80 beats

per minute; however, some variations may occur due to either physiological or pathological reasons. In these animals heart rates were increased as a normal physiological response to the stress caused by parameter recording and interventions.

Table 7: Average heart rate per Group during the treatment days under study

	Control Group	DIB 0.5 g Group	DIB 1,0 g Group	DIB 1.38 g Group	p
Day -7	**93.5 ± 4.7**	**84.5 ± 8.8**	**86.0 ± 7.9**	**81.5 ± 8.2**	**0.7**
Day 0	**84.0 ± 6.0**	**85.0 ± 6.3**	**90.5 ± 7.1**	**90.5 ± 9.3**	**0.9**
Day 9	**97.0 ± 9.8**	**89.0 ± 6.8**	**85.0 ± 7.1**	**95.4 ± 7.8**	**0.7**
Day 16	**90.4 ± 5.2**	**87.0 ± 7.2**	**89.5 ± 7.5**	**91.8 ± 7.5**	**1.0**
P	**0.6**	**1.0**	**0.9**	**0.7**	

Respiratory Rate

No respiratory rate variations were detected in the treatment groups or the days under study (Table 8). The normal values ranged between 10 and 30; all these animals had a normal respiration rate per minute. Some animals had high respiratory rates due to their sanguineous temperament.

Table 8: Average respiratory rate per Group during the treatment days under study.

	Control Group	DIB 0.5 g Group	DIB 1.0 g Group	DIB 1.38 g Group	p
Day -7	**29.5 ± 3.0**	**28.8 ± 3.5**	**34.5 ± 4.2**	**29.0 ± 2.1**	**0.6**

Day 0	**27.0 ± 1.6**	**33.3 ± 2.7**	**34.1 ± 2.8**	**31.9 ± 2.0**	**0.2**
Day 9	**33.0 ± 2.7**	**30.5 ± 2.4**	**33.5 ± 4.4**	**29,.0 ± 2.5**	**0.7**
Day 16	**32.4 ± 3.1**	**37.4 ± 4.5**	**33.3 ± 2.8**	**34.5 ± 2.8**	**0.7**
p	**0.4**	**0.3**	**1.0**	**0.3**	

Complete Blood Count

Blood sampling was conducted as scheduled. Each component of the CBC is mentioned below. The reference values used appear in the Veterinary Merck Manual, Merck & CO.,Inc. Rahway, NJ, USA; 1988. (Annex 7: Reports of the Clinical Analysis Laboratory of the Universidad Católica de Córdoba).

Hematocrit

A difference in hematocrit was observed when the devices were removed (Day 9) among the treatment groups; however, all the values were within the normal range (24 to 46%) (Table 9).

Table 9: Average hematocrit per Group during the treatment days under study

	Control Group	**DIB 0.5 g Group**	**DIB 1.0 g Group**	**DIB 1.38 g Group**	**p**
Day -7	**29.8 ± 1.0**	**28.8 ± 0.8**	**27,4 ± 1,4**	**27.9 ± 0.7**	**0.4**
Day 0	**29.5 ± 1.3**	**29.0 ± 0.7**	**27,3 ± 1,1**	**30.0 ± 0.7**	**0.2**
Day 9	**30.1 ± 0.8** [a b]	**31.5 ± 0.6** [b]	**27,5 ± 0,9** [a]	**31.4 ± 0.9** [b]	**0.007**
Day 16	**30.6 ± 0.7**	**31.0 ± 0.9**	**28,3 ± 1,2**	**30.5 ± 1.3**	**0.3**
p	**0.9**	**0.03**	**0,9**	**0.08**	

Hemoglobin

A difference was detected in hemoglobin levels in the DIB 1.38 g Group, during the treatment; however, all the values were within the normal range of 8 to 156 g/dL. Also, some differences were observed when the devices were removed (Day 9); however, the values are within the normal range (8 to 15 g/dL; Table 10)

Table 10: Average hemoglobin concentration per Group during the treatment days under study.

	Control group	DIB 0.5 g Group	DIB 1.0 g Group	DIB 1.38 g Group	p
Day -7	**12.0 ± 0.4**	**11.7 ± 0.3**	**11.3 ± 0.5**	**11.5 ± 0.3 ^{x}**	**0.6**
Day 0	**11.9 ± 0.4**	**11.6 ± 0.2**	**11.1 ± 0.4**	**12.1 ± 0.2 ^{xy}**	**0.2**
Day 9	**12.2 ± 0.3 ^{ab}**	**12.6 ± 0.2 ^{ab}**	**11.3 ± 0.4 ^{a}**	**12.9 ± 0.5 ^{by}**	**0.02**
Day 16	**12.6 ± 0.3**	**12.4 ± 0.3**	**11.5 ± 0.4**	**12.5 ± 0.4 ^{xy}**	**0.2**
p	**0.6**	**0.03**	**0.9**	**0.06**	

Red blood cells

Red blood cells (erythrocytes) are responsible for carrying oxygen together with hemoglobin from the lungs to the cells in the body, and the CO_2 of cell respiration from the tissues to the lung. The number of red blood cells in millions/mL is a measured parameter (not estimated). Red blood cell count increases with intense training (splenic contraction) and dehydration, and is reduced in the presence of anemia, hemolysis, severe blood loss, chronic inflammation, severe parasitic disease and renal failure. The normal RBC count ranges between 5 and 10 million/mm in adult bovine cattle. All the values obtained were within the normal range (Table 11).

Table 11: Average red blood cell count per Group during the treatment days under study.

	Control group	DIB 0.5 g Group	DIB 1.0 g Group	DIB 1.38 g Group	p
Day -7	**6.2 ± 0.4**	**5.7 ± 0.2**	**5.9 ± 0.2**	**5.9 ± 0.2**	**0.6**
Day 0	**6.2 ± 0.3**	**5.6 ± 0.2**	**6.1 ± 0.1**	**6.2 ± 0.2**	**0.1**
Day 9	**6.2 ± 0.2**	**6.3 ± 0.3**	**5.9 ± 0.2**	**6.5 ± 0.3**	**0.5**
Day 16	**6.4 ± 0.3**	**5.9 ± 0.2**	**6.1 ± 0.2**	**6.3 ± 0.2**	**0.4**
p	**1.0**	**0.1**	**0.8**	**0.4**	

MCV

The mean corpuscular volume (MCV) is the average volume of circulating red blood cells, and is useful to classify different types of anemia. It is measured in femtoliters (fL), and is estimated as follows:

VCM= hematocrit (%) / n° of red blood cells

The normal values range between 40 and 60 fL in adult bovine cattle. All the animals presented values within the normal range, and no differences were observed during the days of the sampling. Some differences were observed on Day 16, among the treatment groups; however, the values were always within the normal range (Table 12).

Tabla 12: Average Mean Corpuscular Volume per Group during the treatment days under study.

	Control Group	DIB 0.5 g	DIB 1.0 g	DIB 1.38 g	p

		Group	Group	Group	
Day -7	**47.7 ± 1.4**	**52.3 ± 1.5**	**46.7 ± 1.9**	**48.0 ± 2.2**	**0.1**
Day 0	**47.8 ± 1.6**	**51.8 ± 1.8**	**46.2 ± 1.3**	**49.2 ± 2.4**	**0.2**
Day 9	**49.6 ± 1.4**	**50.7 ± 2.4**	**48.2 ± 2.2**	**48.7 ± 2.1**	**0.8**
Day 16	**48.8 ± 1.7 [ab]**	**53.1 ± 1.5 [b]**	**46.3 ± 1.3 [a]**	**48.9 ± 2.0 [ab]**	**0.05**
p	**0.8**	**0.8**	**0.8**	**1.0**	

Leukocytes

Some animals had an increased leukocyte count over the normal range of 4 to 12 thousand/mm, in some samples. However, during the days of the study period (Day -7, Day 0, Day 9 or Day 16) none of the animals exhibited a constantly increased leukocyte count during treatment (Table 13). No differences were observed among the treatment groups, and all the leukocyte counts were within the normal range. However, a significant difference was observed in the control group during sampling, but the count was within the normal levels. (Table 13)

Table 13: Average leukocyte count per Group during the treatment days under study.

	Control Group	DIB 0.5 g Group	DIB 1.0 g Group	DIB 1.38 g Group	P
Day -7	**11.4 ± 1.2 [y]**	**11.0 ± 1.5**	**10.7 ± 0.8**	**9.8 ± 1.2**	**0.8**
Day 0	**12.2 ± 0.5 [y]**	**11.7 ± 1.2**	**11.1 ± 0.5**	**10.8 ± 1.7**	**0.8**
Day 9	**12.1 ± 0.4 [y]**	**11.5 ± 0.6**	**11.1 ± 1.0**	**10.6 ± 1.1**	**0.6**

Day 16	**8.2 ± 0.6 [x]**	**8.2 ± 0.3**	**9.6 ± 0.8**	**8.0 ± 0.7**	**0.2**
p	**0.002**	**0.06**	**0.5**	**0.4**	

Granulocytes

Granulocytes are a type of white blood cell including neutrophils, eosinophils, and basophils. They are called granulocytes because they are composed of small granules containing important proteins. Their names come from the typical color they stain when treated with a compound dye; for example, the most numerous granulocytes are neutrophils, whose granules stain a neutral color or almost no color. Eosinophils stain a reddish color (eosinophilia) and basophils have affinity for basic dyes and become bluish. The normal values in bovine cattle range from 25 to 50%. Some differences were seen among the groups on Day 0; but with average values within the normal range. Also, some differences were observed among the treatment days in the Control Group; however, the average values were within the normal range. (Table 14)

Table 14: Granulocyte count in percentage per Group during the treatment days under study

	Control Group	**DIB 0.5 g Group**	**DIB 1.0 g Group**	**DIB 1.38 g Group**	**p**
Day -7	**46.9 ± 4.3 [y]**	**42.5 ± 2.9**	**47.4 ± 4.0**	**43.9 ± 2.4**	**0.7**
Day 0	**27.8 ± 2.3 [x a]**	**34.6 ± 3.3 [ab]**	**40.0 ± 2.8 [b]**	**39.9 ± 1.6 [b]**	**0.006**
Day 9	**37.0 ± 2.1 [xy]**	**36.8 ± 4.3**	**41.0 ± 3.4**	**45.4 ± 2.5**	**0.2**
Day 16	**43.0 ± 2.1 [y]**	**37.6 ± 2.9**	**47.1 ± 2.2**	**41.1 ± 2.8**	**0.08**
p	**0.0004**	**0.4**	**0.2**	**0.4**	

Lymphocytes

These are the most common blood cells in Bovidae, ranging from 2500 and 7500 per uL (45-75% of leukocytes). Some differences were detected during the study period in the Control Group only; however, the average values were within the normal range. Also, differences were observed among the groups, on Day 0, but the average lymphocyte values were within the normal range. (Table 15).

Table 15: Lymphocyte count in percentages per Group during the treatment days under study

	Control Group	DIB 0.5 g Group	DIB 1.0 g Group	DIB 1.38 g Group	p
Day -7	**43.4 ± 4.7 ^{x}**	**49.5 ± 3.6**	**44.8 ± 4.5**	**47.1 ± 3.0**	**0.7**
Day 0	**65.4 ± 2.6 $^{y\ b}$**	**59.5 ± 3.1 ^{ab}**	**53.0 ± 2.9 ^{a}**	**52.0 ± 1.8 ^{a}**	**0.004**
Day 9	**54.1 ± 2.6 $^{x\ y}$**	**55.5 ± 5.5**	**51.5 ± 3.7**	**44.4 ± 3.0**	**0.2**
Day 16	**48.8 ± 1.8 ^{x}**	**55.0 ± 3.5**	**45.8 ± 32.2**	**49.1 ± 2.7**	**0.1**
p	**0.0002**	**0.4**	**0.3**	**0.3**	

Monocytes:

The monocyte count in bovine cattle ranges between 25 and 840 /uL (2 to 7%). Monocytosis is typically present in chronic bacterial infections, chronic fungal processes, immune-mediated conditions, severe hemolytic crisis, and severe tissue necrosis. No differences were seen among the study groups or the treatment days. However, all the values were in the upper limits of normal or outside the upper range (Table 16).

Table 16: Monocyte count in percentages per Group during the treatment days under study.

	Control group	DIB 0.5 g Group	DIB 1.0 g Group	DIB 1.38 g Group	p
Day -7	**9.8 ± 1.0**	**8.0 ± 1.1**	**7.9 ± 0.9**	**9.0 ± 1.3**	**0.6**
Day 0	**6.9 ± 0.6**	**6.4 ± 0.5**	**7.0 ± 0.7**	**8.1 ± 0.7**	**0.3**
Day 9	**7.5 ± 0.8**	**7.5 ± 1.6**	**7.5 ± 0.9**	**10.1 ± 0.8**	**0.3**
Day 16	**8.5 ± 0.5**	**7.4 ± 0.7**	**7.1 ± 0.9**	**9.6 ± 0,9**	**0.09**
p	**0.07**	**0.7**	**0.9**	**0.5**	

Urea

Urea is a relatively simple organic compound produced by mammals in the liver as a final product of protein catabolism. It is one of the most diffusible substances in the body, and is found in all body fluids. It is relatively non toxic, although in high concentrations it denatures proteins and toxic products are formed. The kidneys are responsible for urea clearance though part of it is cleared through the skin, mainly in animals which sweat.

In the presence of renal disorders, such as end-stage and acute kidney failure, urinary tract obstruction, excessive protein destruction as in the case of fever, toxicity or severe sepsis blood urea levels are increased. Also, blood urea levels may increase due to hemoconcentration typically caused by severe vomiting or diarrhea, or in the presence of impaired heart function reducing the renal blood flow. Decreased blood urea levels are uncommon; in theory they may be associated to severe liver disease or protein deficiency. The normal values for bovine cattle range between 15 and 35 mg/dL. Although some differences were observed among the groups when the devices were inserted (Day 0), the values were

within the normal ranges. Also, some differences were found between the DIB 1.0 g and DIB 1.38 g groups during the different days under study, but the values obtained were within the normal parameters for adult bovine cattle (Table 16).

Table 16: Average urea levels per Group during the treatment days under study.

	Control Group	DIB 0.5 g Group	DIB 1.0 g Group	DIB 1.38 g Group	p
Day -7	**15.4 ± 0.4**	**19.4 ± 1.2**	**15.9 ± 0.7 ^{x}**	**17.4 ±1.1 ^{x}**	**0.1**
Day 0	**17.6 ± 0.9 ^{a}**	**19.9 ± 1.4 $^{a\,b}$**	**22.1 ± 1.4 $^{y\,b}$**	**18.4 ± 0.9 $^{y\,a\,b}$**	**0.05**
Day 9	**19.5 ± 1.8**	**19.5 ± 1.3**	**19.9 ± 1.3 $^{x\,y}$**	**22.1 ± 0.6 $^{x\,y}$**	**0.4**
Day 16	**16.0 ± 1.7**	**18.0 ± 1.9**	**16.7 ± 1.0 ^{x}**	**16.1 ± 1.1 ^{x}**	**0.8**
p	**0.1**	**0.9**	**0.002**	**0.0007**	

Creatinine

Creatinine is found in the body mainly in the form of high energy phosphate. It is a source of energy in the muscles. Creatinine is a very diffusible substance, uniformly distributed in the water body content, and cleared from plasma by glomerular filtration.

When studying creatinine clearance it is important to underline that the serum creatinine levels are not affected by exogenous creatinine in food, age, gender, exercise or diet. Therefore, high creatinine levels occur in the presence of impaired kidney function. Blood creatinine levels and urea nitrogen levels provide the same information for the diagnosis and prognosis of impaired kidney function. Differences were found between the control group and the DIB 1.0 g group in the different days of the study (Table 17). Some values were over the range for adult bovine cattle from 0.6 to 1.8 mg /dL, but some authors have found normal

values of 2.6±0.3 mg/dl in crossbred zebu cows (1979, P. Fasano de Magnífico, E. Otaiza V. y V. Cumare). Hemoglobin concentration, ureic nitrogen levels and creatinine in dairy bovine cattle in the center-western region of Venezuela, Agronomía Tropical 29(3): 231-249), the highest value published has been a creatinine concentration of 3.4±0.08 mg/dl (1970 ROUBICEK, C.B., RA Y, D.E. Y HALE. W.H. (Blood creatinine and uric acid concentrations in unsupplemented range cattle. J. Anim. Sci. 30,675-679) who measured those values in 710-day old Hereford males.

Table 17: Average creatinine levels per group during the treatment days under study.

	Grupo Control	DIB 0.5 g Group	DIB 1.0 g Group	DIB 1.38 g Group	p
Day -7	**2.3 ± 0.2 ^{y}**	**2.3 ± 0.1**	**2.1 ± 0.1 ^{y}**	**2.0 ± 0.2**	**0.4**
Day 0	**1.6 ± 0.1 ^{x}**	**1.9 ± 0.1**	**1.6 ± 0.1 ^{x}**	**1.7 ± 0.1**	**0.06**
Day 9	**1.6 ± 0.1 ^{x}**	**1.9 ± 0.1**	**1.7 ± 0.1 $^{x\,y}$**	**1.9 ± 0.1**	**0.1**
Day 16	**2.0 ± 0.1 $^{x\,y}$**	**2.2 ± 0.1**	**2.1 ± 0.1 ^{y}**	**2.0 ± 0.1**	**0.2**
p	**0.0002**	**0.4**	**0.003**	**0.2**	

Total protein levels

Ions, and in a smaller proportion proteins, are the main contributors of plasma osmotic pressure. However, the constant decrease in protein osmotic pressure is vital for the cardiovascular system. Two large groups of proteins are found: albumins and globulins, separated by simple chemical procedures , and the A-G ratio is obtained by determining the number of each group. Blood albumin and globulins, except for some gamma globulins, are

synthesized in the liver. Therefore, any process affecting the albumin synthesis will lead to a decrease in the A-G ratio. Antibody production may cause certain changes in the gamma globulin concentration; however, the change is more qualitative than quantitative.

An increased total protein level may be due to dehydration, which typically features hemoconcentration caused by vomiting and diarrhea, also by an increased globulin level in the absence of dehydration, such as in the case of advanced liver disease (cirrhosis), chronic infection and some neoplasms. A decrease in the total protein level is always due to a low albumin level, accompanied by an increased globulin level or a globulin level which is lower than the decreased albumin level. Therefore, the A-G ratio decreases.

This may be due to: albumin urine loss caused by nephrosis, plasma protein loss due to bleeding, insufficient protein intake, impaired liver function to produce albumin due to hepatitis or liver cirrhosis.

It has been proven that total protein levels may decrease during the different reproductive physiological states in cows (Study of the metabolic profile in dairy cattle in warm weather, one month before calving and in three different stages of nursing, N. B. ANDRADE DE SABOGAL, M. G. RIVERA GAONA, G. TORRES MORENO, registered in the research line of cattle production system , Ibagué (Tolima), Code 16 - Act No. 7. May 1994).

A low blood protein level leads to a reduction in plasma colloidal osmotic pressure which in turn may lead to edema. No differences were observed among the groups or the treatment days; however, the averages indicated low total protein levels outside the normal ranges of 6.2 to 8.2 g/dL, Table 18).

Table 18: Average total protein levels per Group during the treatment days under study.

	Control group	**DIB 0.5 g Group**	**DIB 1.0 g Group**	**DIB 1.38 g Group**	**p**

	Control Group	DIB 0. 5 g Group	DIB 1.0 g	DIB	p
Day -7	**5.9 ± 0.3**	**5.6 ± 0.2**	**5.6 ± 0.2**	**5.5 ± 0.3**	**0.8**
Day 0	**5.5 ± 0.2**	**5.6 ± 0.3**	**6.0 ± 0.2**	**5.7 ± 0.4**	**0.6**
Day 9	**5.8 ± 0.2**	**5.8 ± 0.1**	**5.7 ± 0.2**	**6.0 ± 0.3**	**0.7**
Day 16	**5.7 ± 0.2**	**5.7 ± 0.2**	**5.8 ± 0.2**	**5.8 ± 0.2**	**0.9**
p	**0.8**	**0.9**	**0.6**	**0.6**	

GLUTAMIC OXALACETIC TRANSAMINASE (GOT - AST)

This hialoplasmatic enzyme is typically found in the cells; the highest concentration is found in muscle fibers, which accounts for the high levels when muscle necrosis occurs.

GOT catalyzes the transference of the a-amino group of aspartic acid to α-ketoglutaric acid. It is vital to measure it in large animals for it may be suggestive of muscular lesion or liver necrosis. This enzyme is considerably increased in soft tissue myopathies. The increased values must be related to another enzyme called CPK since in conditions involving muscles both AST and CPK are increased, for example in monensin poisoning. The normal GOT values in bovine cattle ranges from 45 to 110 U/L.

Table 19: Average GOT levels per Group during the treatment days under study.

No differences were observed among the groups during the days under study. Significant differences were seen in the DIB 0.5 g group during the treatment days, but the values are within the normal range.

			Group	1.38 g Group	
Day -7	**109.4 ± 6.0**	**109.8 ± 5.8 [y]**	**115.4 ± 14.1**	**110.3 ±8,.5**	**1.0**
Day 0	**104.1 ± 10.6**	**91.6 ± 5.5 [xy]**	**97.0 ± 14.4**	**90.4 ± 7.1**	**0.8**
Day 9	**87.0 ± 5.4**	**79.5 ± 4.1 [x]**	**97.8 ± 12.4**	**85.3 ± 5.1**	**0.4**
Day 16	**90.6 ± 3.1**	**104.6 ± 8.5 [y]**	**109.8 ± 13.6**	**88.3 ± 6.8**	**0.3**
P	**0.08**	**0.007**	**0.7**	**0.07**	

GLUTAMIC PYRUVIC TRANSAMINASE (GPT- ALT)

This enzyme catalyzes the transference of an a-amino Group of alanine to α-ketoglutaric acid, and is found in the hialoplasma of cells. There is a linear relationship between liver GPT and the animal´s weight. GPT measurement is almost specific to the dog and cat´s liver, and is almost of no value for diseases affecting bovine cattle or horses. It is very high in liver necrosis, a very stable enzyme, and when frozen it may be stored for a long time.

The presence of jaundice does not prevent enzyme measurement, but hemolysis should be avoided. Liver conditions with high GPT levels are malignant neoplasms, cirrhosis and hepatitis. No differences were observed among the groups or treatment days. All the animals exhibited values within the normal range (7 to 40 U/L, Table 20).

Table 20: Average Glutamic pyruvic transaminase levels per Group during the treatment days under study

	Control group	DIB 0.5 g Group	DIB 1.0 g Group	DIB 1.38 g Group	p
Day -7	**37.6 ± 3.5**	**35.9 ± 4.5**	**31.1 ± 2.2**	**38.3 ± 3.5**	**0.5**
Day 0	**31.3 ± 2.2**	**35.1 ± 4.1**	**24.9 ± 3.2**	**31.8 ± 2.9**	**0.2**
Day 9	**27.9 ± 2.5**	**30.6 ± 2.7**	**28.0 ± 3.5**	**33.6 ± 3.6**	**0.5**
Day 16	**29.5 ± 4.3**	**34.0 ± 3.4**	**26.8 ± 3.6**	**31.4 ± 4.2**	**0.6**
p	**0.2**	**0.8**	**0.3**	**0.5**	

Creatinephosphokinase (CPK):

Also called creatine kinase (CK), this enzyme is typically found in striated muscles, and increases in muscle conditions featuring degeneration or necrosis.

CPK is an enzyme found in high concentrations in muscle tissue, both skeletal and cardiac muscle, and in lower concentrations in other tissues. It includes three enzymes: MM, MB, y BB, and is used for the diagnosis of acute myocardial infarction (AMI) and as a reliable measurement of muscle inflammatory conditions.

CPK values are a useful lab tool for the diagnosis and follow up of myopathies. CPK levels usually rise in the presence of myopathies. No differences were observed among the groups during the study period. Significant differences were seen in the DIB 0.5 g group during the treatment days, but the values were within the normal range. (Necrosis of the muscles and heart – infarction), muscle injuries, conditions involving the CNS and muscle fatigue. The normal values range between 44 and 230 U/L. No differences were found among the treatment days. Differences were observed among the groups on Day 16. The average levels

on Day 16 for the DIB 0.5 g and DIB 1.0 g groups were higher than the normal ranges for two cows exhibited high CPK levels on that day (Table 21).

Table 21: Average CPK levels per Group during the treatment days under study.

	Control group	DIB 0.5 g Group	DIB 1.0 g Group	DIB 1.38 g Group	p
Day -7	**181.6 ± 16.3**	**257.1 ± 50.7**	**217.9 ± 25.9**	**240.0 ± 45.4**	**0.5**
Day 0	**227.4 ± 46.5**	**286.8 ± 54.0**	**187.5 ± 17.1**	**256.3 ± 38.2**	**0.4**
Day 9	**224.1 ± 36.0**	**231.8 ± 18.0**	**310.4 ± 76.1**	**247.6 ± 16.7**	**0.5**
Day 16	**202.0 ± 20.3** [a]	**470.9 ± 99.1** [b]	**314.8 ± 87.2** [a b]	**208.3 ± 14.4** [a]	**0.03**
p	**0.7**	**0.05**	**0.3**	**0.7**	

Fibrinogen

Fibrinogen is an acute phase plasma protein produced and released into the blood flow by the liver in response to an inflammatory process. Also, it is part of the coagulation cascade (factor I) and is a precursor of fibrin to repair damaged tissue and favor the migration process of inflammatory cells, fibroblasts and endothelial cells. The measurement of fibrinogen concentrations may be used as an optional tool for monitoring the animal´s health status. The information obtained is relevant to either confirm o rule out any presumptive diagnosis.

No differences were observed among the treatment days in the groups. Differences were found in the control Group, the DIB 1.0 g and DIB 1.38 g groups in the different days studied. All the values were within the normal range from 100 to 600 mg/dL (Table 22).

Table 22: Average fibrinogen levels per Group during the treatment days of the study.

	Control Group	DIB 0.5 g Group	DIB 1.0 g Group	DIB 1.38 g Group	p
Day -7	**243.9 ± 17.0 x**	**264.6 ± 17.8**	**253.8 ± 25.0 x**	**258.5 ± 17.6 x**	**0.9**
Day 0	**298.9 ± 15.4 y**	**269.3 ± 16.3**	**286.5 ± 8.2 x y**	**291.0 ± 7.5 x y**	**0.4**
Day 9	**302.1 ± 6.7 y**	**303.1 ± 6.1**	**299.4 ± 2.9 x y**	**312 ± 8.6 y**	**0.5**
Day 16	**289.1 ± 9.1 x y**	**303.0 ± 5.9**	**313.9 ± 6.8 y**	**305.3 ± 8.2 y**	**0.2**
p	**0.01**	**0.7**	**0.03**	**0.01**	

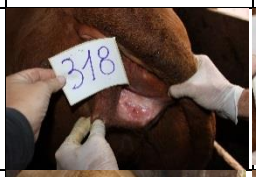
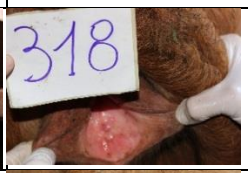
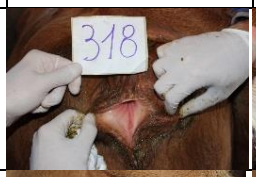
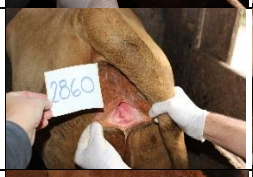
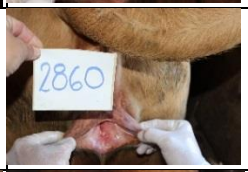
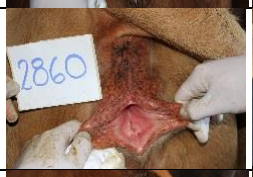
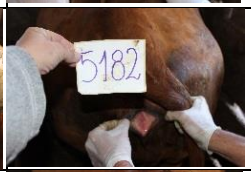
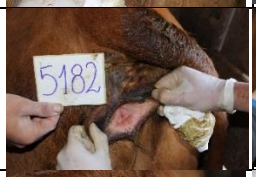
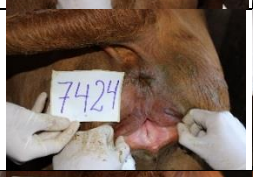
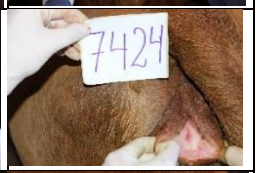
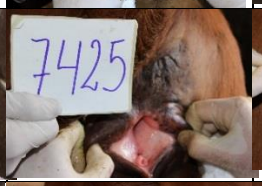
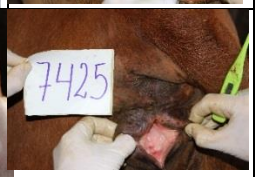
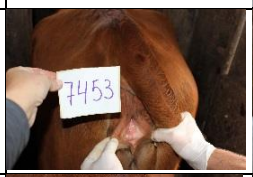
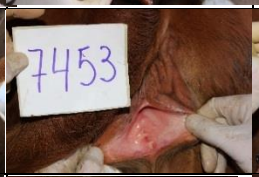
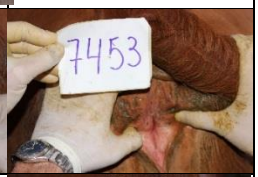
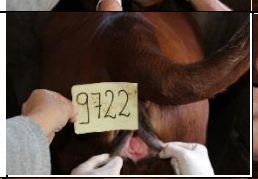
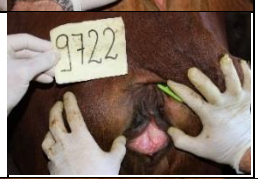
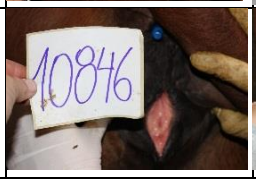
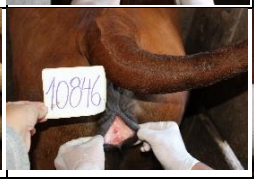
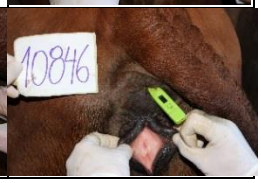
Vaginal Mucosa, Cervix and Uterus:

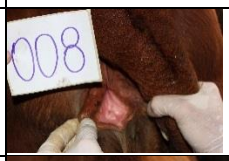
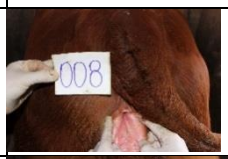
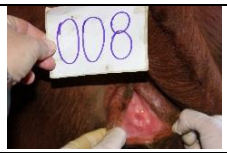
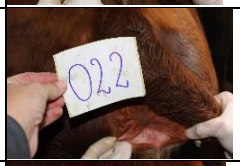
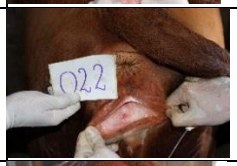
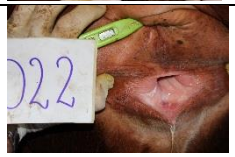
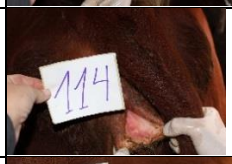
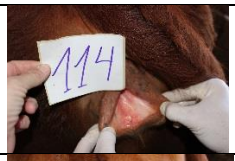
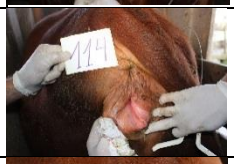
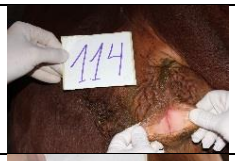
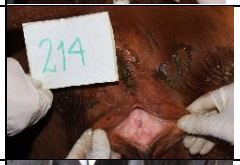
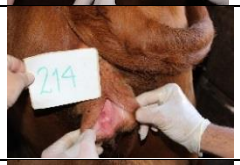
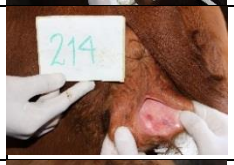
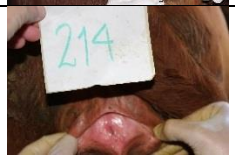
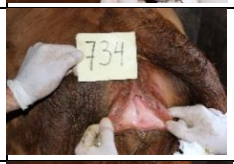
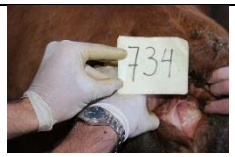
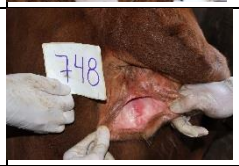
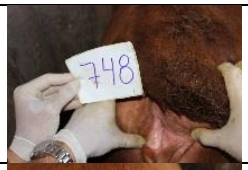
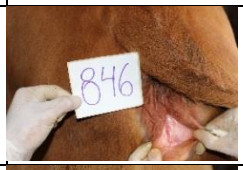
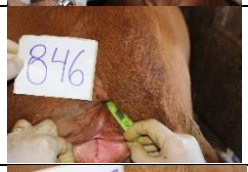
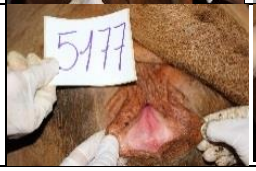
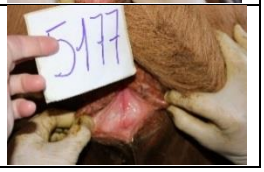
Vestibular vaginal mucosa: no abnormality or change was seen related to the status in the vaginal mucosa and cervix 7 days before insertion (Day -7).

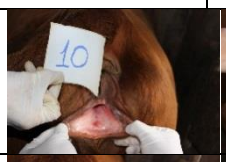
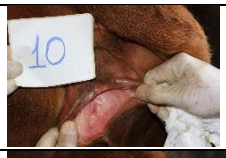
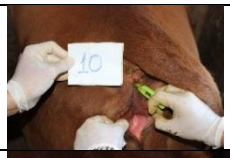
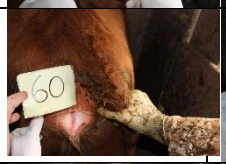
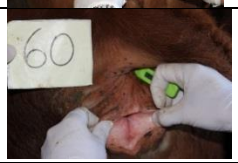
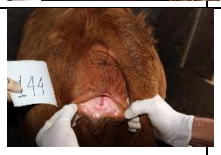
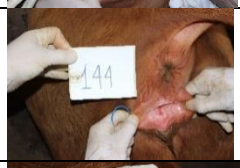
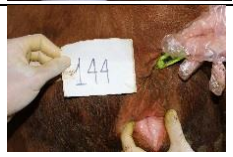
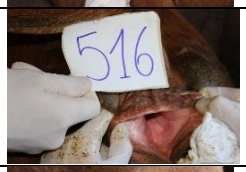
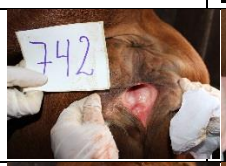
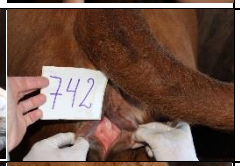
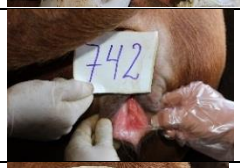
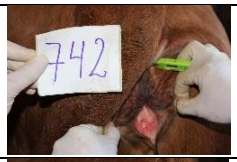
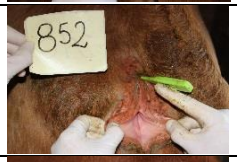
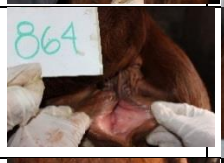
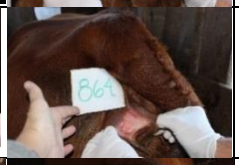
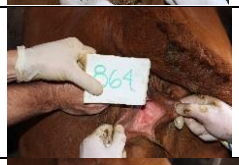
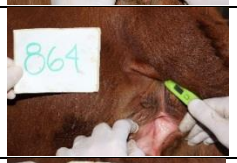
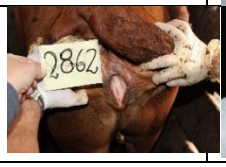
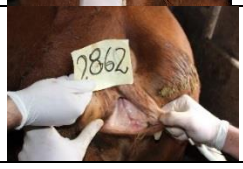
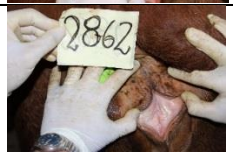
No changes were observed in the vaginal mucosa and cervix when the devices were inserted (Day 0), nor when the devices were removed (Day 9), nor 7 days after the devices had been removed. The mucosa membranes were normal and light pink. Some animals exhibited a darker pink or reddish mucosa with vascularization and discharge for they were in either proestrus or estrus.

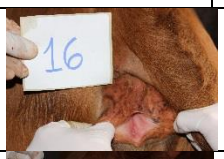
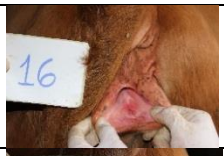
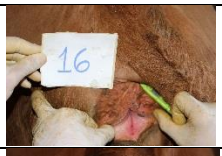
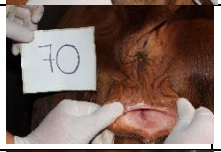
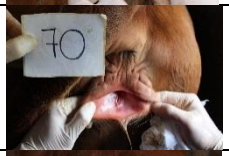
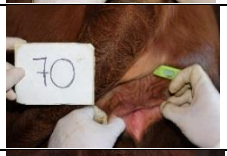
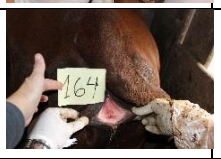
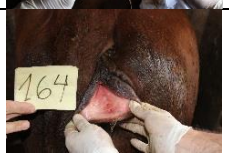
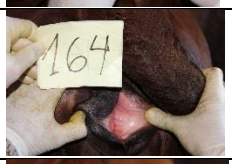
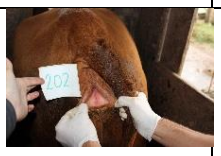
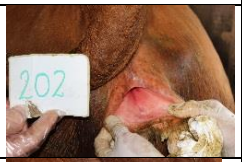
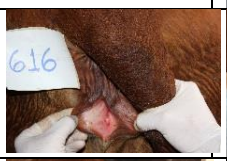
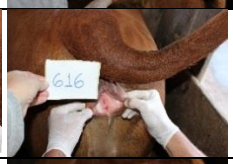
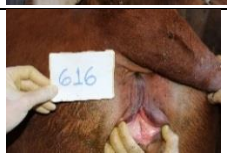
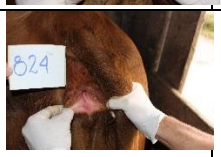
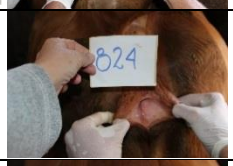
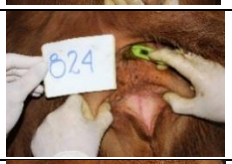
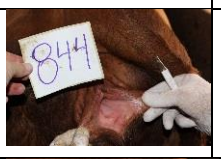
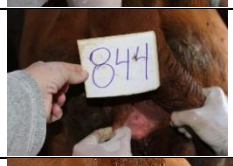
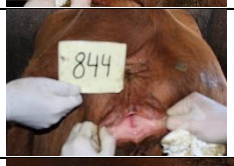
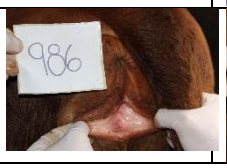
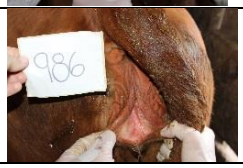
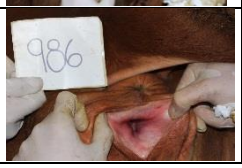
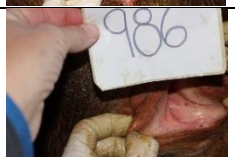
Below are the photos of each cow, of the vagina and cervix per day and treatment group.

UNIVERSIDAD CATÓLICA DE CÓRDOBA
Universidad Jesuita

Control Group	Day -7	Day 0	Day 9	Day 16
318				
2860				
5182				
7424				
7425				
7453				
9722				
10846				

DIB 0.5 g Group	Day -7	Day 0	Day 9	Day 16
8				
22				
114				
214				
734				
748				
846				
5177				

DIB 1.0 g Group	Day -7	Day 0	Day 9	Day 16
10				
60				
144				
516				
742				
852				
864				
2862				

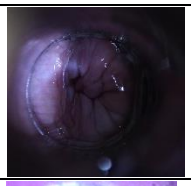
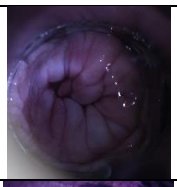
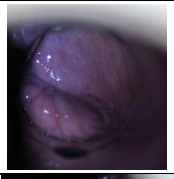
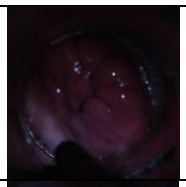
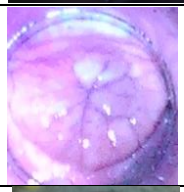
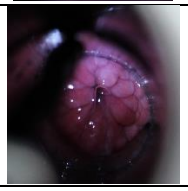
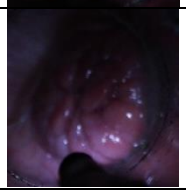
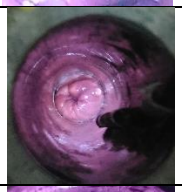
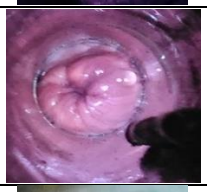
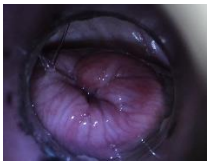
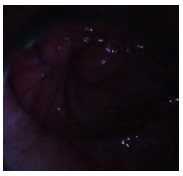
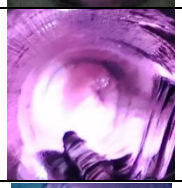
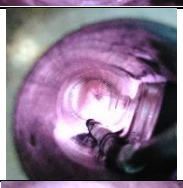
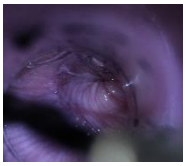
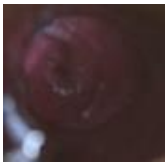
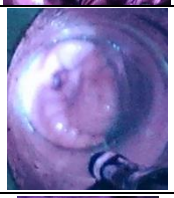
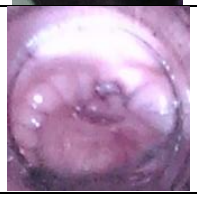
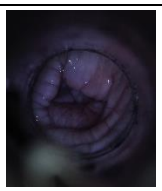
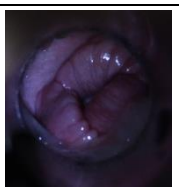
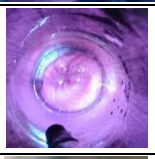
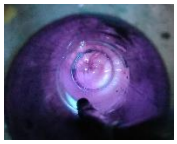
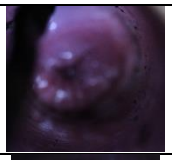
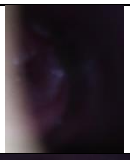
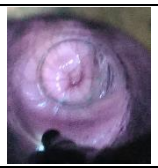
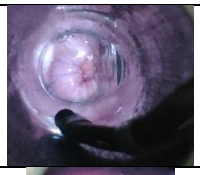
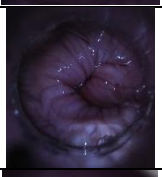
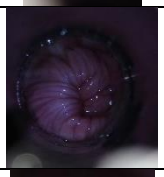
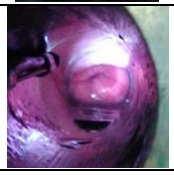
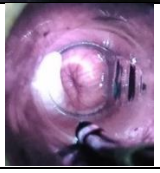
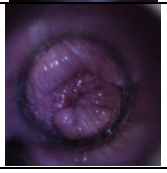
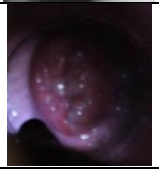
DIB 1.38 g Group	Day -7	Day 0	Day 9	Day 16
16				
70				
164				
202				
616				
824				
844				
986				

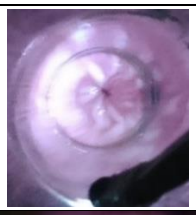
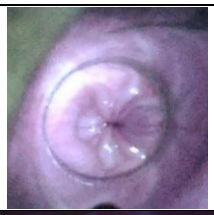
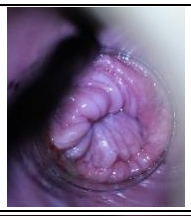
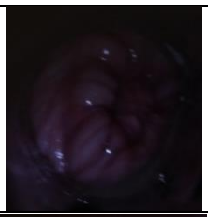
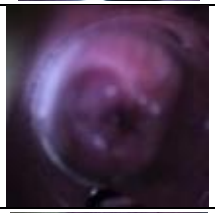
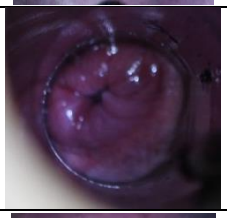
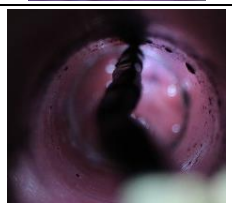
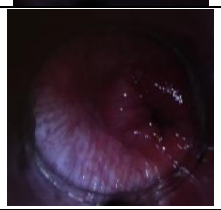
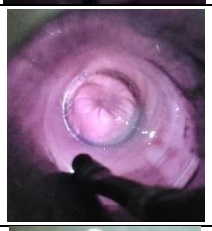
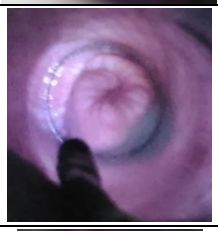
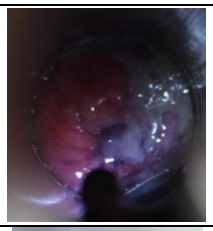
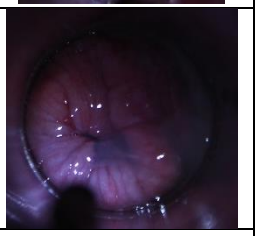
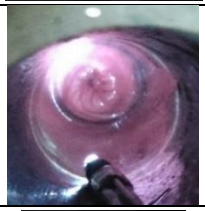
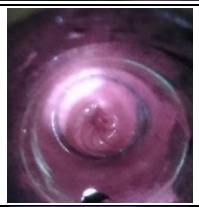
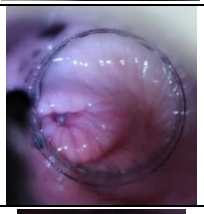
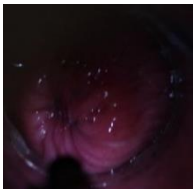
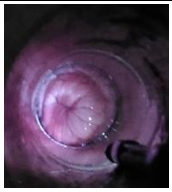
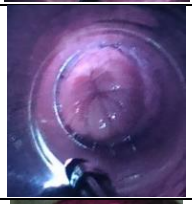
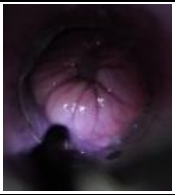
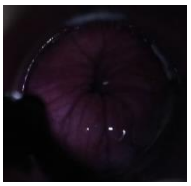
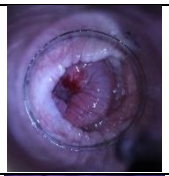
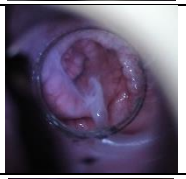
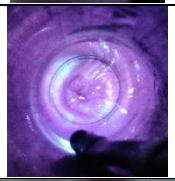
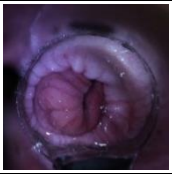
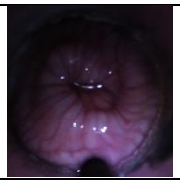
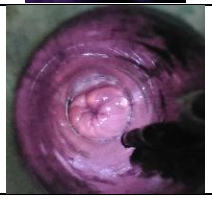
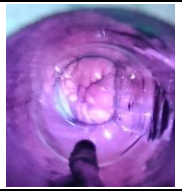
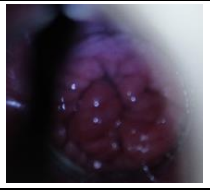
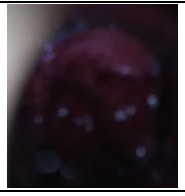
Vaginoscopy of the Vaginal mucosa: the vaginoscopy showed vaginal mucosa changes according to hormonal changes, during proestrus and estrus it was hyperemic and during anestrus it was anemic. Apart from these physiological cases no hyperemia due to inflammatory processes, such as acute vaginitis, was observed.

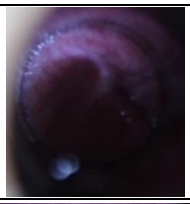
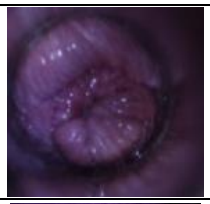
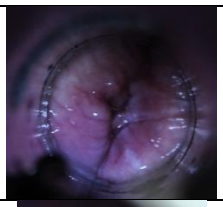
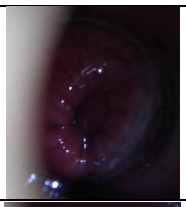
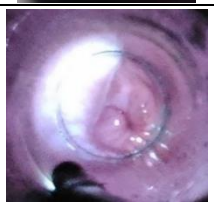
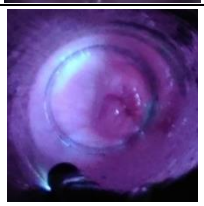
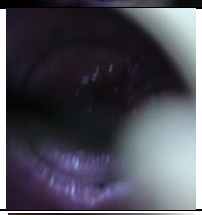
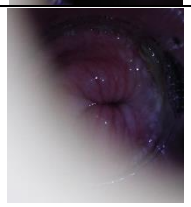
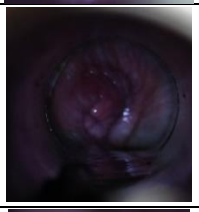
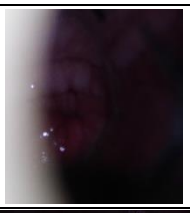
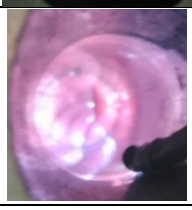
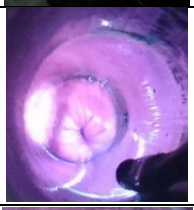
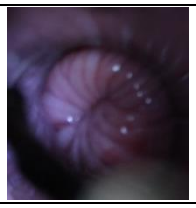
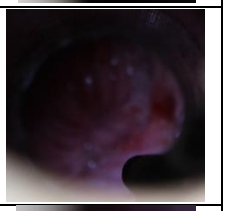
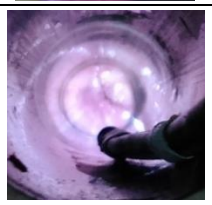
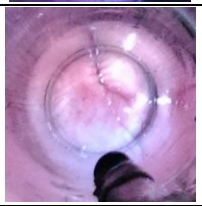
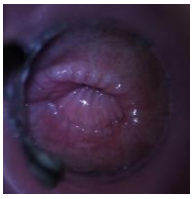
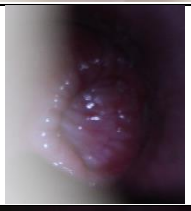
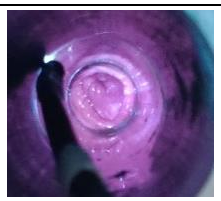
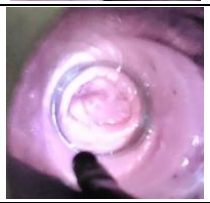
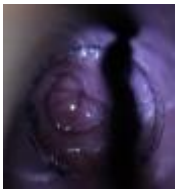
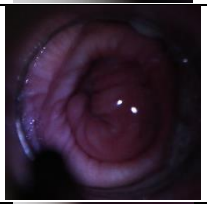
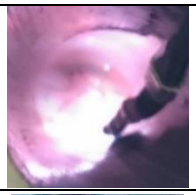
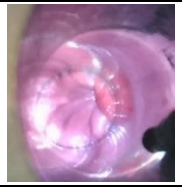
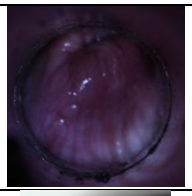
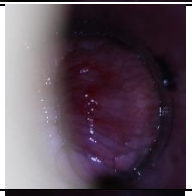
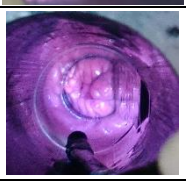
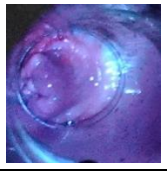
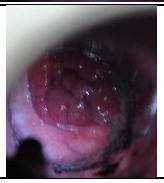
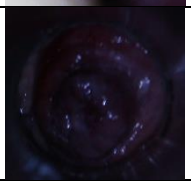
The humidity rate was also related to the physiological status of the animals, cows in proestrus or estrus had an increased glandular secretion, so their vagina looked wet and bright, whereas animals in diestrus presented a dry vaginal mucosa.

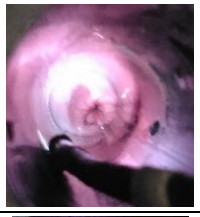
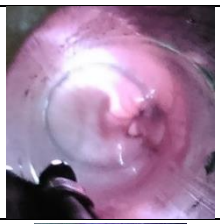
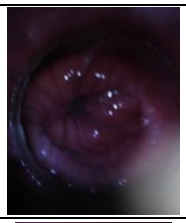
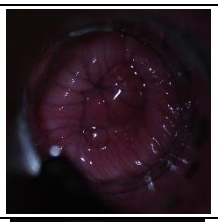
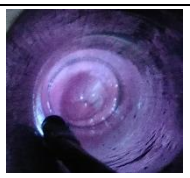
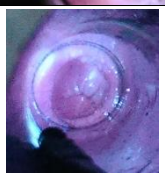
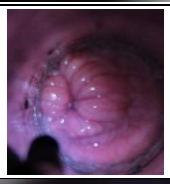
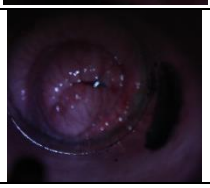
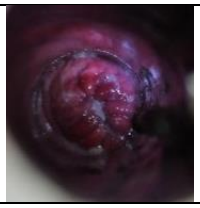
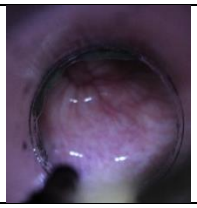
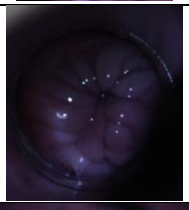
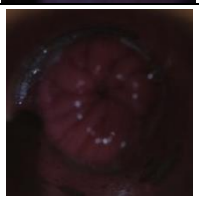
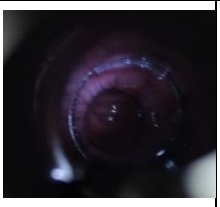
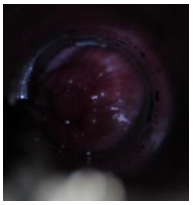
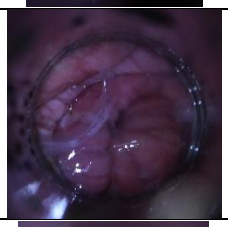
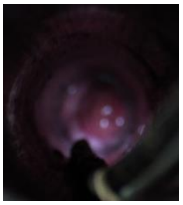
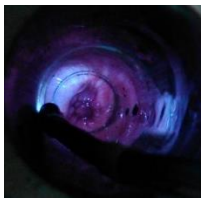
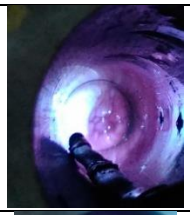
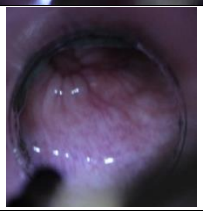
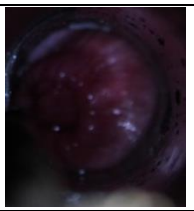
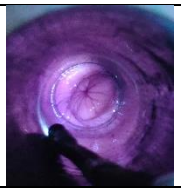
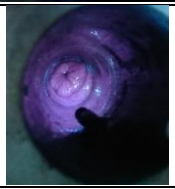
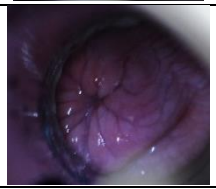
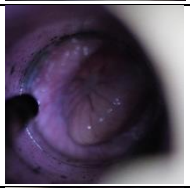
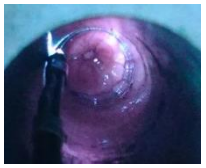
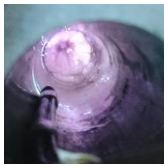
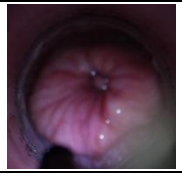
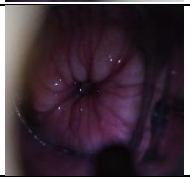
Vaginoscopy of the cervix: the cervix also presented changes depending on the physiological status of the animals. Animals in diestrus had a cone-shaped ostium uteri, similar to a closed rosebud. Cows in estrus or proestrus had a deformed or widened cervix, changes were sometimes observed in the vaginal floor.

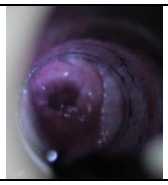
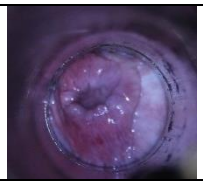
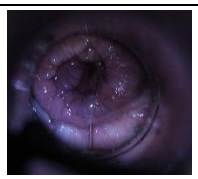
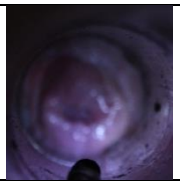
No abnormal vaginal discharge was seen on any of the study days (day -7, Day 0, Day 9 and Day 16). Moreover, on examination no abnormal changes were seen in the uterus 7 days before the device was inserted, as compared to examinations conducted on the following days. (Day 0, Day 9 and Day 16)

Control Group	Day -7	Day 0	Day 9	Day 16
318				
2860				
5182				
7424				
7425				
7453				
9722				
10846				

DIB 0.5 g Group	Day -7	Day 0	Day 9	Day 16
8				
22				
114				
214				
734				
748				
846				
5177				

DIB 1.0 g Group	Day -7	Day 0	Day 9	Day 16
10				
60				
144				
516				
742				
852				
864				
2862				

DIB 1.38 g Group	Day -7	Day 0	Day 9	Day 16
16				
70				
164				
202				
616				
824				
844				

986				

Ultrasound of the uterus and ovaries: all the liquid and parenchymal structures were observed, and depending on the physiological status of the animal normal ovaries as to shape and size were seen, with the typical follicular and luteal structure (follicles and corpus luteum). No abnormal formations were observed in the uterus, cysts, tumors, endometritis, polyps, etc. This data appear in the ovarian follow up records annexed in this report. (Annex 5)

Conclusion

This TAS study provides information about the safety of three different intravaginal devices with progesterone (P4): DIB (1g of Progesterone), DIB 0.5 (0.5 g of Progesterone) and DIB 1.38 (1.38 g of Progesterone) used with the target species, bovine females. These devices are manufactured by Syntex SA, Argentina.

This study included 32 animals whose body conditions and weight did not change during the treatment period. No changes in temperature, coccygeal pulse or heart and respiratory rate were detected. No changes in the complete blood count (CBC) or blood chemistry panel were seen during the treatment period.

The devices did not cause any changes in the vulvar o vaginal mucosa either while in use or after being removed. No changes were seen in the cervix or inside the uterus. No abnormalities were observed in terms of ovarian dynamics.

This report is submitted on August 19th, 2014

Dolores Moreno, DVM, PhD.
Catholic University of Córdoba
School of Agricultural Sciences
dmorenob74@gmail.com