

Plasma progesterone levels in animals treated with intravaginal devices
containing different progesterone concentrations

Experiment 1 – Final Report

Objective:

To determine progesterone plasma levels in non-ovariectomized cows treated with bovine intravaginal devices (DIB) with different progesterone concentrations.

Hypothesis:

No significant differences were observed in plasma progesterone levels in beef cows treated with intravaginal devices containing different progesterone concentrations used for the first time.

Materials and Methods:

Professionals responsible for the study:

The experiment was conducted by veterinarians from Universidad Católica de Córdoba, Dr. Mariano Avilés, Dr. Leonel Alisio and Dr. Dolores Moreno. Dr. Lucas Cutaia (Technical manager at Syntex SA. Dr. Lucas Cutaia (Syntex SA) was responsible for the management, design and supervision of the study.

Place and date: the study started on February 17th, 2014 (day 12 of treatment) and finished on March 9th, 2014 (day 8 of treatment) at the Santa Julia Experimental Premises of the Universidad

Católica in Córdoba located 20 km from the city of Cordoba, 10 km from Monte Cristo. The weather information is annexed at the end of the study (Annex 1).

Laboratory logbook: a page numbered logbook was drafted including all the events recorded during the study, such as temperature, rainfall, time of food supply, application-related issues, animal disease, etc. (Annex 2). Also, all the field spreadsheets (Annex 3) and the digital records will be submitted to complement this report.

Animals: 14 adult non suckled beef cows calved in 2013 were included in the study. The cows were San Ignacio (Hereford x AngusxTuli) with an average weight of 380 kg. The average body condition score (BCS) was 2.5 (CC from 1 to 5) at treatment initiation, and the cows were kept in a feed lot setting at Santa Julia Farm of the Universidad Católica in Córdoba, were given a controlled diet (30kg of corn silage, 3 kg of ground corn, 0.5 kg of soybean expeller per animal per day.) Both weight and body condition score (BCS) were recorded at treatment initiation (see Photo 1). All the cows were individually identified with a tag, and were individually photographed at treatment initiation (this information is annexed in digital format).



Photo 1: Typical animal included in the study, average BCS 2.5 and 380 kg average weight, individually identified with a tag.

Study product: DIB, DIB 0.5 and DIB 1.38

Treatment groups: 3 groups were included in the study, DIB Group 1g (n=5), DIB Group 1.38 g (n=5) and DIB Group 0.5g (n=4). The treatment schemes appear in Table 1.

The animals were presynchronized to be on about day 10 of the cycle on a 2cc dose of PGF (Cicalse FL, batch CV, 1013), on day 12 am and another 2 cc dose of PGF pm (Cicalse DL, batch CV 1013). On that same day, a transrectal ultrasound was performed to plot the ovarian status in an ultrasound spreadsheet.

On day 0, after ultrasound, the animals were randomly selected for 1 of the 3 treatment options, considering their body conditions score and ovarian structures, and so they received a 1 g DIB (Syntex SA, batch IND 1538 E 12/13 Y V 12/16; blue tail , n =5, Photo 2), a 1.38 g DIB, used

(without a batch number for it was manufactured for this particular field test; green tail, n =5 , Photo 3) or a 0.5 g DIB (DUC 1051 E11/13 YV 11/15, grey tail, n =4 Photo 4). The devices were removed on Day 7, were washed and conditioned for the next experiment (Annex 6).

The treatment groups were as follows:

DIB 1.0 g Group (n=5): animals: 144, 214, 60, 10 and 202.

DIB 1.38 g Group (n=5): animals: 16, 824, 2860, 864 and 2860

DIB 0.5g Group (n=4): animals: 9722, 986, 164 and 70.

Table 1. Treatment Groups

	DIB 1.0 g Group (n=5)	DIB 1.38 g Group (n=5)	DIB 0.5 g Group (n=4)	Blood	US
Day -12	PGF AM and PM			X	X
Day -1	PGF AM and PM			X	X
Day 0	DIB 1.0 g	DIB 1.38 g	DIB 0.5 g	X	X
Day 1				X	
Day 2				X	
Day 3				X	
Day 4				X	
Day 5				X	
Day 6				X	
Day 7	DEVICES REMOVED			X	X

Day 8				X	
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Photo 2: Typical cow included in the DIB 1g Group. The photo shows the blue tail.



Photo 3: Typical cow in the DIB 1.38g Group. The photo shows the green tail.



Photo 4: Typical cow in the DIB 0.5g Group. The photo shows the grey tail.

Sample collection

Blood samples were obtained by puncturing all the cows in the jugular vein on Day 12, and then from Day 1 until Day 8 (Photo 5) in order to determine plasma progesterone levels. The samples were always obtained between 9 and 10 AM in heparin tubes (Photo 6) and immediately after collection were centrifuged (Photo 7), and plasma was frozen at -20°C , by duplicate (A or B, Photo 8), and duly identified according to the cow number, treatment day, date and either A or B (Photo 9).



Photo 5: Jugular puncture for sample collection on Day-12 and from Day 1 until Day 8.



Photo 6: Blood samples in heparin tubes identified for each cow.



Photo 7: Samples centrifuged at 10.000 rpm for 10 minutes

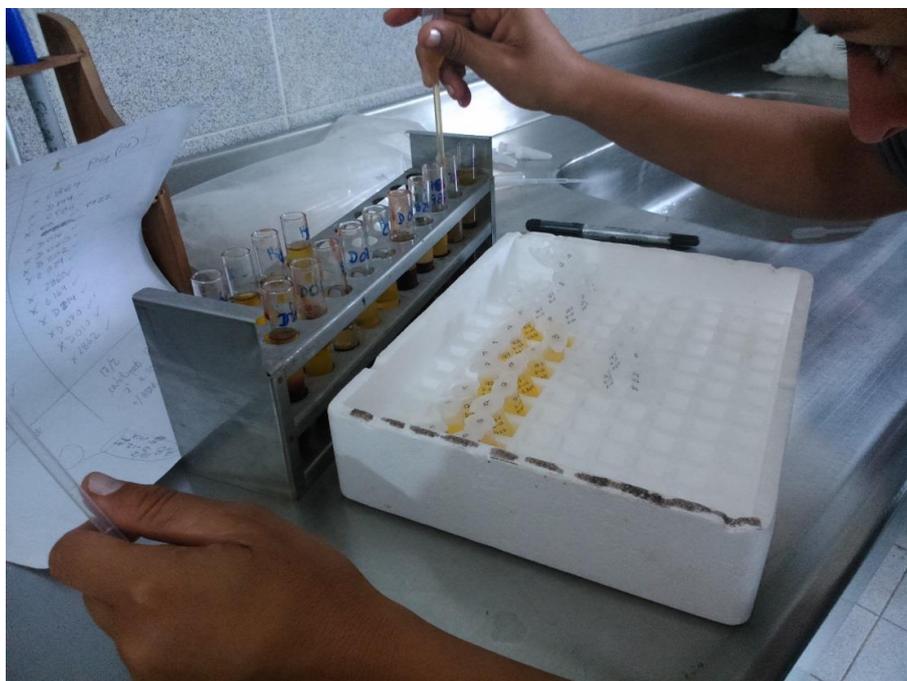


Photo 8: Plasma eppendorf tubes by duplicate (A or B) to be frozen.

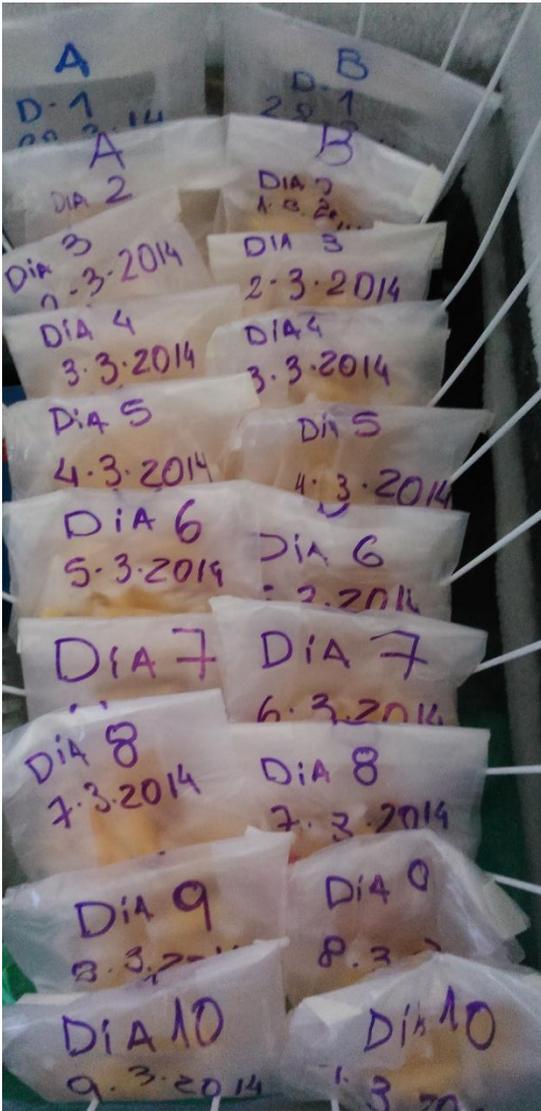


Photo 9: Frozen and duly identified samples. The eppendorf tubes with the cow number, treatment day and date. Samples frozen in bags by duplicate A or B, and labeled (treatment day and date).

The samples were processed at Laboratorio LACE in the city of Cordoba by means of electrochemoluminescence immunoassay using the diagnostic kit for Progesterone II (Roche Alecsys Cobas, catalog number 121145383 122, Annex), and the B samples were frozen to be sent to Syntex as controls. The intra-assay variation quotient was 1.16%, and sensitivity reached 0.03 ng/mL.

Ultrasound: transrectal ultrasound was conducted (Chison D 600 Vet Digital, using a 7.5 MHz transducer) on Day 12 to monitor the ovarian status and select the animals for the study (Photo 10). On Days 1, 0 and 7 ultrasound was performed to determine the ovarian structures present, which was plotted in a spreadsheet for ovarian follow up. (Photo 11).



Photo 10: Ultrasound performed on Day 12, Day 1, Day 0 and Day 7.

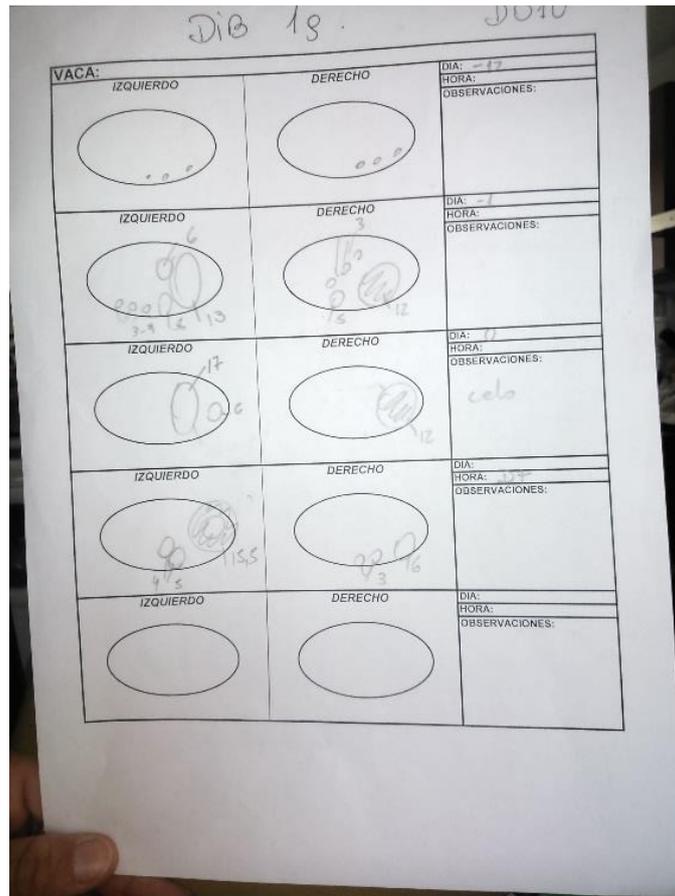


Photo 11: Spreadsheet used for ovarian control on Days 12,-1, 0 and 7.

Statistical Analysis

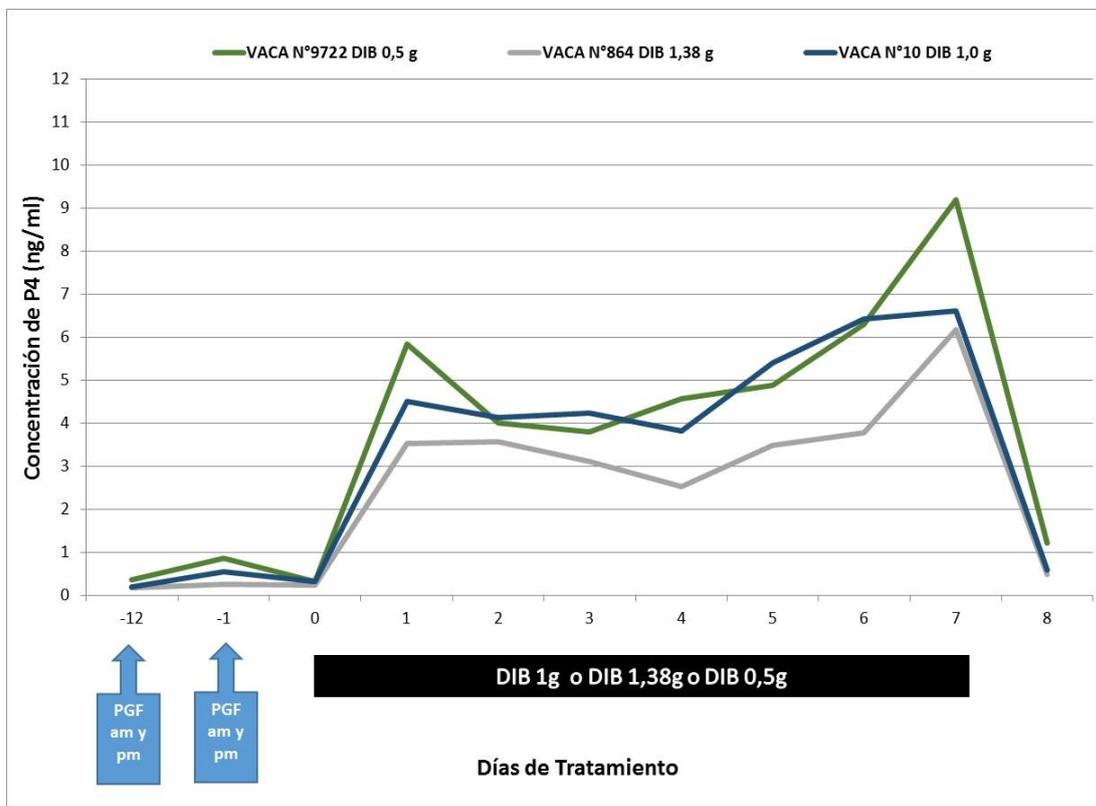
Plasma progesterone levels were obtained by the average area under the curve (AUC) for each Group, and then compared by the ANOVA test in order to determine the total progesterone release level for each device. ANOVA was performed for the measurements (Infostat, UNC) to compare the hormonal levels among the study groups and the Group*Day interaction.

Results

Two cows, one in the DIB 0.5g Group (cow n° 9722) and another cow in the DIB 1.0g Group (cow n°10) ovulated during the treatment, between Day 1 and Day 2. This occurred because cows were

approximately on Day 10 of the cycle when the PGF dose was administered on Day -1 (AM and PM). Therefore the ovulation mechanism in these cows was triggered before DIB insertion for they exhibited a large dominant follicle (proestrus). In the DIB 1.38 g Group, one of the cows (number 864) developed a CL (corpus luteum) at treatment initiation for no CL regression by PGF on Day -1 (am and PM) had occurred probably because it was a young CL as a result of late ovulation after PGF administration on Day -12.

For this reason cow number 9722, cow number 10 and cow number 864 were excluded from AUC analysis since they exhibited a CL during treatment (Graph 1).



Graph 1: cows with a CL during treatment, excluded from further analysis to compare AUCs in the 3 treatment groups.

Two cows, one in the DIB 1.38 g Group (cow number 2860) and one cow in DIB 1.0 g Group (cow n° 60) lost their devices on Day 6 of treatment and the devices were reinserted on the same day;

however, the values of the samples collected on that day are below average for the cows in each group. For this reason, and in order to include them in the analysis the average recorded the day before and the day after were considered for each cow, and that value was recorded for that day. Also, an extreme value was found in one cow from the DIB 1.0 g Group 25 ng/mL (cow n°144) on Day 1, which was corrected considering the average observed on that day in the other cows in the DIB 1.0 g Group.

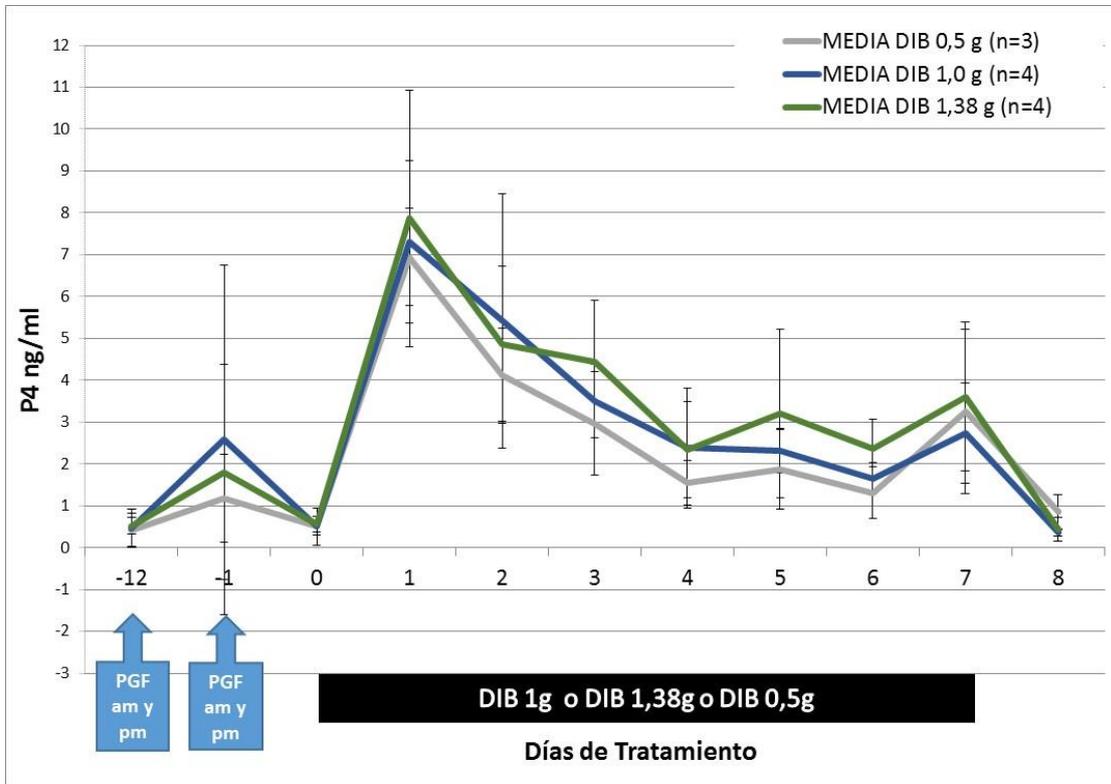
The average AUC was 2324.4 ng for the DIB 1.0 g Group, 2290.9 ng for the DIB 0.5 g Group and 2351.3 ng for the DIB 1.38 g Group. The differences were not statistically significant (P=0.91). No differences (P=0.15) were observed among the levels of the 3 treatment groups or Group *Day interaction (P=0.99; Graph 2).

Variance Analysis

Variable	N	R ²	R ²	Aj	CV
Progesterone	99	0.75	0.66	47.97	

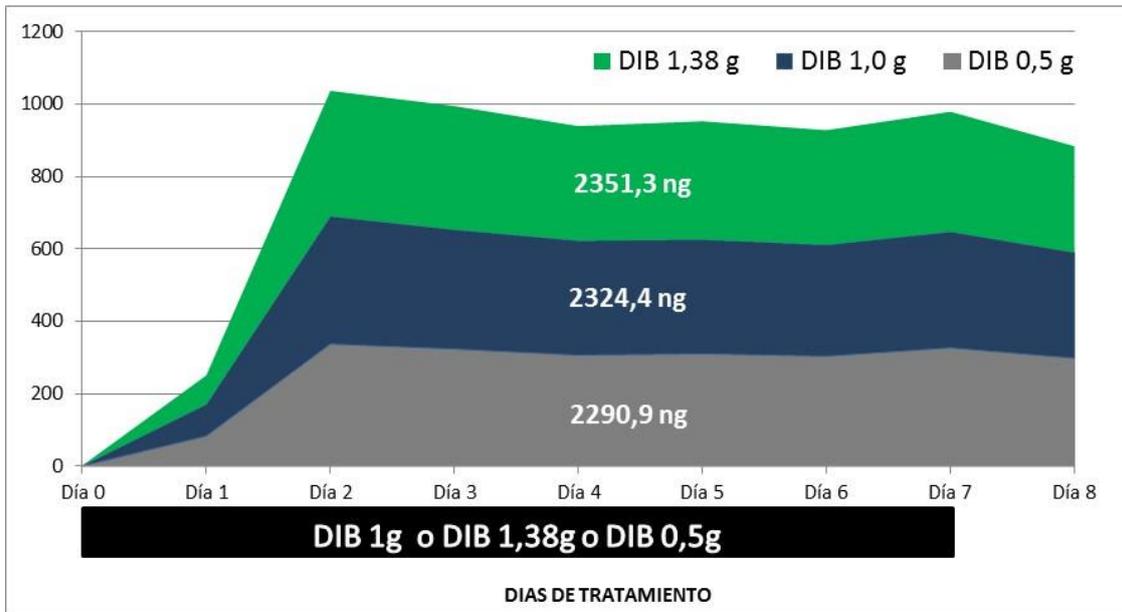
Variance Analysis Table (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	435.80	26	16.76	8.28	<0.0001
Group	7.77	2	3.88	1.92	0.1541
Time	404.85	8	50.61	25.01	<0.0001
Group*Time	9.49	16	0.59	0.29	0.9959
Error	145.70	72	2.02		
Total	581.50	98			



Graph 2: Plasma progesterone levels in the treatment groups: DIB 1.0g, DIB 1.38g and DIB 0.5g.,

Moreover, a graph including the cumulative AUCs for each device from Day 0 (device insertion) until Day 8 (Graph 3) was plotted.



Graph 3: Cumulative AUC of the plasma progesterone levels for the treatment groups, DIB 1.0g, DIB 1.38g and DIB 0.5g.

Conclusions:

We may conclude that no differences were detected in plasma progesterone levels during the 7-day treatment in non-ovariectomized cows treated with first use 0.5 g DIB; 1.0 g DIB and 1.38 g DIB. The values obtained in this study are consistent with the values reported in the literature.