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The dairy cow: Understanding
the sustainable production

Protocols J-Synch with and without eCG in Brown Swiss and crosses whith Bos indicus cows in the Ecuadorian Amazons

Protocolo J-Synch con y sin eCG en vacas Brown Swiss y sus cruzas con Bos Indicus en la amazonía ecuatoriana

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Abstract

The incorporation of protocols with prolonged proestrus in extreme conditions has allowed to improve pregnancy. The objective was to evaluate the results of the protocol with prolonged proestrus called J-Synch with and without eCG, on follicular dynamics and pregnancy percentage in dual-purpose cows in the Ecuadorian Amazon. The project was developed of the Province of Pastaza - Ecuador, from October 2018 to October 2019. The study used 448 multiparous dual purpose Brown Swiss cows with calves and their crosses (*Bos indicus*). Four treatments were used, the cows were artificially inseminated at a fixed time on day nine T1: J-Synch+ eCG+ estrus FTAI 60 hours (n=120); T2: J-Synch+ eCG+ without estrus+ GnRH FTAI 72 hours (n=118); T3: J-Synch+ estrus FTAI 60 hours (n=103); T4: J-Synch+ without estrus + GnRH + FTAI 72 hours (n=107). The pregnancy rate was 55% (T1), 49% (T2), 51% (T3) and 50% (T4) without showing significant differences. Follicular development and corpus luteum diameter showed significant differences ($p \leq 0.05$). It is concluded that the J-Synch treatments plus eCG at 60 hours and 72 hours had different behavior in follicular development and in the size of the corpus luteum. The pregnancy percentage did not

differ between treatments. Although, there was a 44.5% more probability of getting a cow pregnant with T1 compared to T2. Furthermore, pregnancy would increase by 22.5% applying T1 compared to T3, and pregnancy with T3 would be 18% higher if compared to T2.

Keywords: Follicular dynamics, pregnancy, progesterone, Fixed Time Artificial Insemination

Resumen

La incorporación de protocolos con proestro prolongado en condiciones extremas ha permitido mejorar la concepción. Por lo tanto, el objetivo de esta investigación fue evaluar los resultados del protocolo con proestro prolongado denominado J-Synch con y sin eCG, sobre la dinámica folicular y porcentaje de preñez en vacas doble propósito en la Amazonía Ecuatoriana. El trabajo se efectuó en la provincia de Pastaza - Ecuador, desde octubre del 2018 a octubre del 2019. Se utilizaron 448 vacas multíparas en producción con cría al pie de las razas Brown Swiss y sus cruzas (*Bos indicus*). Se trabajó con cuatro tratamientos, las vacas fueron inseminadas artificialmente a tiempo fijo en el día nueve T1: J-Synch + eCG +Celo IATF 60 horas (n=120); T2: J-Synch+eCG +Sin Celo+ GnRH IATF 72 horas (n=118); T3: J-Synch+ Celo IATF 60 horas (n=103); T4: J-Synch+ Sin Celo+ GnRH +IATF 72 Horas (n=107). La tasa de preñez fue del 55% (T1), el 49% (T2), el 51% (T3) y el 50% (T4) sin mostrar diferencias significativas. El desarrollo folicular y el diámetro del cuerpo lúteo mostraron diferencias significativas ($p \leq 0,05$). Se concluye que los tratamientos J-Synch más eCG a las 60 horas y las 72 horas tuvieron diferente comportamiento en el desarrollo folicular y en el tamaño del cuerpo lúteo. El porcentaje de preñez no difirió entre los tratamientos, aunque existió un 44,5% más probabilidad de quedar preñada una vaca con el T1 en comparación con el T2. Además, que aumentaría un 22,5% la preñez aplicando el T1 en comparación con el T3, y se lograría un 18% mayor de preñez con el T3 si se compara con el T2.

Palabras clave: Dinámica follicular, preñez, progesterone, inseminación artificial a tiempo fijo

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1 Introduction

Brown Swiss cow production systems and their crossings with *Bos indicus* are common in the humid tropical region, with an average daily milk production per cow of 3 to 9 liters, the duration of lactation from 120 to 180 days and a delivery interval of 18 to 24 months (Rodriguez et al., 2015).

These reproductive parameters are generated by multiple factors, among them environmental (high temperatures and humidity), physiological and zootechnical management. In the Amazon region, producers need to improve the reproductive outcomes in cows for meat and milk (Moyano et al., 2015).

J–Synch protocol has suffered a number of variations, without altering its initial form as: an intramuscular dose of Estradiol Benzoate and the placement of progesterone through an intravaginal device to synchronize the beginning of a new follicular wave with lower insertion time of the device with progesterone for 6 days and no longer than 7 or 8 days, applying a GnRH as ovulation initiator at 72 hours after the removal of the device, along with the FTAI, to ensure a more durable proestrus (De la Mata and Bó, 2012).

Yánez et al. (2017), showed the pregnancy results of two FTAI protocols by inseminating double purpose cows from the Ecuadorian Amazon at 60 and 72 hours of having removed the intravaginal device impregnated with progesterone, where the J-Synch and eCG 60 hour protocol had the best pregnancy rate compared to the J-Synch and eCG 72 hour protocol. Cedeño et al. (2019) compared the follicular and luteal dynamics of an alternative J-Synch proestrus prolongation treatment of 7 days of progesterone in *Bos indicus* heifers that received fixed time artificial insemination in Manabí Province-San Vincente, concluding that the extension of one more day of progesterone in the 7 day J-Synch treatment does not alter the characteristics of the dominant ovulation follicle and the time of ovulation in relation to the 6 day J-Synch protocol, although in both prolonged proestrus protocols, the interval to ovulation is higher than in animals treated with the conventional protocol with Estradiol Cypionate as an ovulation inductor. Castellanos et al. (2019) conducted a study in Etzatlán, Jalisco, Mexico, with 211 cows (90 Brahman cows and 121 F1 Brahman $\times$ Swiss Brown cows) without breeding with more than 180 postpartum days and a body condition of 3 to 4; on Day 6, 105 cows were randomly assigned to the J-Synch Group and the remaining cows (106) were assigned to the Traditional Group, the two groups with eCG, finding no significant differences ($p \geq 0.05$) in the pregnancy percentage among the groups. In addition, it showed no significant differences ($p \geq 0.05$) in the pregnancy percentage of the breed or the bull used.

Based on the findings of the application of J-Synch, (Bó et al., 2018a) proposed that heifers that manifest an earlier estrus could be inseminated earlier without affecting the pregnancy percentage, but it would be necessary to determine the optimal time to perform FTAI in those that do not show estrus. A research was carried out, in which 1,283 Angus and Hereford cross heifers were treated with the J-Synch protocol and heifers were painted at the base of the tail when the progesterone device was removed. Heifers received GnRH/FTAI at 60 or 72 h, regardless the paint removal. Pregnancy was higher in those who showed estrus before FTAI than in those who did not, no matter the time of insemination (53.6%, 542/1012 vs. 45.0%, 122/271, respectively, $P \leq 0.05$).

The practical implication of this result is that when large herds are synchronized, the device could be removed in the afternoon of the 6th day and the FTAI begins at 60 h (day 9 a.m.) on all heifers with altered paint; and those with the paint intact could be separated to receive GnRH at the time of artificial insemination on a fixed time on the afternoon of the 9th (i.e. around or after 72 hours).

The incorporation of protocols with prolonged proestrus has allowed to increase the pregnancy rates in cows with anestrus, with elevated open days, with foot breeding in tropical climates. However, the J-Synch protocol is still required to continue to be validated under conditions in the Ecuadorian Amazon. The objective was to evaluate the results of the protocol with prolonged proestrus called J-Synch, with and without eCG, in follicular behavior and the pregnancy percentage in Brown Swiss cows and their crosses with *Bos indicus* in the Ecuadorian Amazon.

2 Materials and Methods

The research was carried out in Pastaza, province of Ecuador, starting from October 2018 to October 2019. In the Amazon, the precipitation is 4000 – 5000 mm/year, high humidity and heat with temperatures ranging between 18 and 24 °C. Producers own farms of 50 ha average on uneven land, using *Axonopusscoparius* (Gramalote) (González-Marcillo et al., 1997).

448 cows of the Brown Swiss breeds and their *Bos indicus* crosses were used, which were fed by a grazing system (Carrera Durazno et al., 2015), from 90 to 132 days of postpartum, from 34 to 65 months old, with a body condition from 2.5 to 3 (scale 1 to 5, Ben et al. (2002)), and a weight between 350 and 380 kg, and were selected by the presence of a follicle > 8 mm in diameter in their ovaries by means of ultrasonography. Finally, 0.50 ml brown Swiss semen straws previously analyzed in FTAIs were used.

2.1 Treatments

The long term proestrus resistant synchronization protocol called J-Synch (De la Mata and Bó, 2012) was used, which is modified by adding eCG and performing the FTAI at 60 and 72 hours of having removed the intravaginal device. The total cows were randomly divided into two groups: Group 1 with the addition of eCG and Group 2 without eCG. In turn, these two groups were subdivided into two more groups, one that is performed with FTAI at 60 hours and another with FTAI at 72 hours after the intravaginal device had been removed. Schematically represented.

T1: J-Synch + eCG + *Estrus* - IA 60 hours (n=120)
T2: J-Synch + eCG + *Without estrus* + GnRH - FTAI 72 hours (n=118)
T3: J-Synch + *Estrus* – IA 60 hours (n=103)
T4: J-Synch+ *Without estrus* + GnRH - FTAI 72 Hours (n=107)

The distribution of treatments (T) was completely at random, each of which carried out four repetitions (*T1: 30, 30, 30 and 30 (120), T2: 29, 29, 30 and 30 (118), T3: 25, 26, 26 and 26 (103) T4: 26, 27, 27 and 27 (107)* cows with each of the treatments).

For the conduction of the research, on day 0 a first ultrasound reproductive evaluation was carried out on each of the 306 cows to analyze ovarian status and observe if animals meet the proposed parameters to be included in this research; additionally, an intravaginal device with progesterone (DIB 0.5 gr) was placed and 2 mg of BE per IM was applied; on day 6 cows were divided into groups according to their treatments as follows:

2.2 Treatment 1

References: On day 0, the first ultrasound evaluation was performed to analyze the ovarian status and BE (2 mg of Estradiol Benzoate) was applied via IM together with an intravaginal DIB device 0.5 g. On day 6 the device was removed with the administration of $PGF_{2\alpha}$ (500 μg of Cloprostenol) plus the application of eCG (400UI of equine chorionic Gonadotropin) and divided into two groups: *T1* (120 cows) and *T2* (118 cows) were measured with ultrasonography of follicular development at the onset of the proestrus, a paint marker was applied at the base of the tail to observe the presence of preFTAI (Artificial insemination at a fixed time) (Figure 1). The animals that presented estrus within 60 hours were inseminated at 60 hours *(T1)* and those that did not, at 72 hours with the addition of GnRH (100 μg gonadorelin acetate) *(T2)*. On day 9 or end of the proestrus, the ultrasonogram was performed to see the behavior of the follicles. Following this, 60 hours after having removed the intravaginal device, the time of ovulation was determined by ultrasonogram every 12 hours in the two treatments. On day 7 post insemination, the size of the luteal body was measured by ultrasound and quality was measured through a serum sample, and progesterone levels (ng/ml) were analyzed. The gestation diagnosis was performed 35 to 40 days after FTAI by ultrasound (Veterinary ultrasound Ibex-pro with linear probe 5 MH in V mode).

2.3 Treatment 2

References: On day 0, the first ultrasound evaluation was performed to analyze the ovarian status and BE (2 mg of Estradiol Benzoate) was applied via IM along with an intravaginal 0.5 g DIB device. On day 6 the device was removed with the administra-

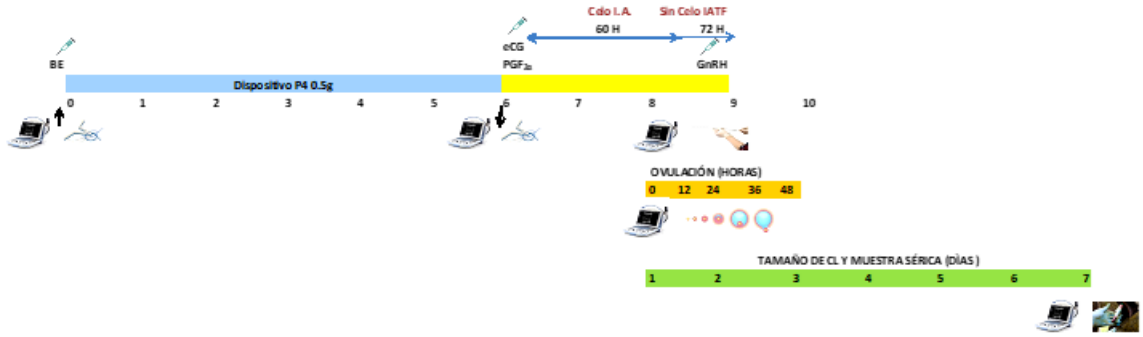


Figure 1. T1: J-Synch +eCG+ Estrus – I.A. 60 hours. Without estrus I.A.T.F. 72 Hours + GnRH

tion of $PGF_{2\alpha}$ (500 μg of Cloprostenol) and divided into two groups: *T3* (103 cows) and *T4* (107 cows) a measurement with ultrasonography of follicular development was performed at the beginning of the proestrus, a paint marker was applied at the base of the tail to observe the presence of preFTAI (Fixed time artificial insemination). The animals that showed estrus before 60 hours were inseminated at 60 hours *(T3)* and those that did not, at 72 hours plus the addition of GnRH (100 μg gonadorelin acetate) *(T4)*. On day 9 or end of the proestrus, the ultrasonogram was performed to see the behavior of the follicles. Following this, 60 hours after having removed the intravaginal device, the time of ovulation was determined by ultrasonogram every 12 hours in the two treatments. On day 7 post insemination, the size of the luteal body was measured by ultrasound and quality was measured through a serum sample, and progesterone levels (ng/ml) were analyzed. The gestation diagnosis was performed 35 to 40 days after FTAI by ultrasound (Veterinary ultrasound Ibex-pro with linear probe 5 MH in V mode) (Figure 2).

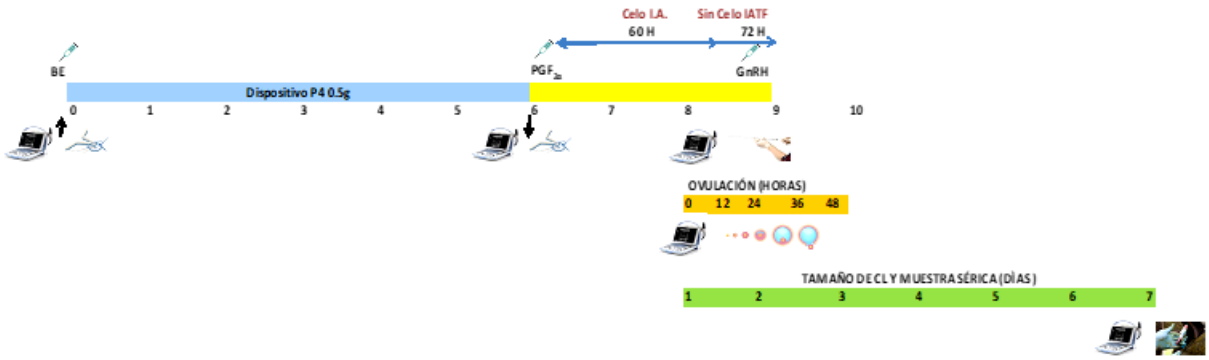


Figure 2. T2: J-Synch+ Estrus – I.A. 60 hours. Without estrus I.A.T.F. 72 Hours + GnRH

Frozen/thawed semen was used for the FTAI using the technique described by Bernardi et al. (2011), of a single bull of proven fertility, also before using it an analysis was done in the Biotechnology Laboratory of the Center for Research, Postgraduate and Amazonian Conservation and were inseminated by the same technician.

Estruses were observed through two behaviors: depainted in the bases of the tail and vaginal mucus (egg white), this observation was made before the ultrasound and FTAI started. In addition, estrus detection started 24 hours after the device was removed, twice a day (morning and afternoon) on days 7, 8 and 9.

2.4 Follicular and luteal dynamics

The follicular development was evaluated with an ultrasound on day six when the intravaginal device of progesterone was removed, also at the time of conducting the FTAI (60 and 72 hours of having removed the intravaginal progesterone device) with the aim of monitoring the ovary behavior and at the end of the estrus. In addition, on day seven after the insemination the measurements of the size of the luteal body are measured with the ultrasound, using what is recommended by Kastelic et al. (1990), on two measures (height-width in mm) of each structure (luteal body or follicle) to obtain the average of both variables.

In order to determine the timing of ovulation (disappearance of the larger diameter follicle), the follicle behavior was evaluated ultrasonically, observing the follicular development. It was performed in a smaller group of cows T1 and T2 = 24; T3 and T4 = 18 due to the difficulties in accessing or staying at different times in the establishments used; for this purpose, an ultrasound was performed 12 hours after the FTAI, then the animals that had not yet registered ovulation had another ultrasound at 24 hours, and the animals that had not yet ovulated until this moment were subjectively determined to ovulate $>$ 24 hours after the FTAI, which was reconfirmed with the presence of CL 7 days after the FTAI.

Gestation was determined through ultrasonography between 40 to 45 days of FTAI (Ibex Pro 2011 and Lyte, USA, L6.2 transducer with a linear probe of 5.0 MHz)

2.5 Variables analyzed

- Estrus detection (presence or absence)
- Follicular development in mm
- Time of ovulation (hours)
- Luteal body size post-ovulation in mm
- Pregnancy: presence or absence
- Time: day 6 and day 8

2.6 Statistical analysis.

A Multiple Logistic Regression model was used for the adjustment to study the pregnancy (presence or absence) depending on the different protocols, days, follicular development in mm, size of the post-ovulation luteal body in mm. This model allows to take into account the binary nature of the response variable. The model interpretation was performed by analyzing the estimated Odds Reasons, along with their confidence intervals. For statistical analyses the JMP version 5.0 program for Windows was used.

3 Results

3.1 Pregnancy percentage

Out of the 448 inseminated cows, 230 (51.3%) cows got pregnant and 218 (48.6%) cows did not get pregnant. The pregnancy percentage of the T1, T2, T3 and T4 protocols showed no significant differences (P$\geq$0.7989) (Table 1).

Table 1. The percentage of pregnancies in Brown Swiss cows and their crosses subjected to J-Synch treatments with and without eCG at 60 and 72 hours of FTAI.

Treatments	N° animals	Pregnant animals	% pregnancy
T1	120	(66/120)	55
T2	118	(58/118)	49
T3	103	(53/103)	51
T4	107	(53/107)	50
Total	448	(230/448)	51.3

3.2 Estrus manifestation

There was 51% (230/448) of estrus in the total of animals for both treatments (Table 2). The percentage of animals with a presence of estrus in the J-Synch treatment with eCG (T1 and T2) was 52.1% (124/238) and in the J-Synch treatment without eCG (T3 and T4) was 50% (106/210) without significant differences (P$\geq$0.05).

In the cows that presented estrus, the percentage of pregnancy in the J-Synch protocol with eCG + I.A. 60 hours was 55% (66/120) *T1* and J-Synch without eCG + I.A., at 60 hours *(T3)* it was 54% (39/72), which showed no significant difference, as well as cows without the presence of estrus + GnRH + FTAI 72 hours that were 49% (58/118) *T2* and 49% (67/138) *T4*, respectively (P$\geq$0.05).

Table 2. Estrus in Brown Swiss cows and their crosses subjected to J-Synch treatment with eCG and without eCG.

Treatments	N° animals	With estrus	Without estrus
J- Synch con eCG	238	124 (52.1%)	114 (47.8%)
J-Synch sin eCG	210	106 (50.5%)	104 (49.5%)
TOTAL	448	230 (51.3%)	218 (48.6%)

3.3 Follicular dynamics at different times

The follicular size when the device was removed showed significant differences ($p \leq 0.05$) with a larger diameter for *T2* (9.7 ± 0.1 mm); in the same way, the size of the follicle initiation of artificial insemination showed significant differences ($P \leq 0.05$) with a larger diameter for *T4* (13.2 ± 0.1 mm) and when the estrus ended, the size of the follicle showed significant differences ($P \leq 0.05$) with a larger diameter for *T3* (13.9 ± 0.2 mm).

Table 3. Follicular dynamics (mm) in the four protocols used in Brown Swiss cows and their crosses. Standard ave-rages and errors.

	Follicle size in mm		
Treatment Moment	**Once removed the device**	**Artificial insemination**	**At the end of the estrus**
T1	9.3 ± 0.1 ab	12.8 ± 0.1 a	13.0 ± 0.2 b
T2	9.7 ± 0.1 a	12.1 ± 0.1 b	12.8 ± 0.2 b
T3	9.3 ± 0.1 ab	12.2 ± 0.1 b	13.9 ± 0.2 a
T4	9.1 ± 0.1 b	13.2 ± 0.1 a	12.5 ± 0.8 ab

Note: different letters in each column indicate significant differences ($P \leq 0.05$).

3.4 Moment of ovulation

Ovulation, since the removal of the device, was higher ($F=18.2$; $P \leq 0{,}001$) or the treatment J-Synch with eCG (T1 and T2) (94.5 ± 0.9) hours than for the J-Synch group without eCG (88.0 ± 1.1) hours. Figure 3 shows the ovulation response and pregnancy rate registered in percentage at 84, 96 and 108 hours of FTAI in Brown Swiss cows and their crosses under the J-Synch treatment with eCG. Figure 4 shows the ovulation response and pregnancy rate registered in percentage at 84-96 and 108 hours of FTAI in Brown Swiss cows and their crosses with the J-Synch treatment without eCG.

Table 4. Ovulation after 24 hours of FTAI in Brown Swiss cows and their crosses according to J-Synch treatments with and without eCG.

Treatments	N° animals	Hours of ovulation	% ovulation
J-Synch with eCG	24	94.5 ± 0.9	51.0
J-Synch without eCG	18	88.0 ± 1.1	48.5
TOTAL	42	91.25 ± 1.0	50.0

Figure 3. Ovulation response and pregnancy rate with J-Synch treatment with eCG.

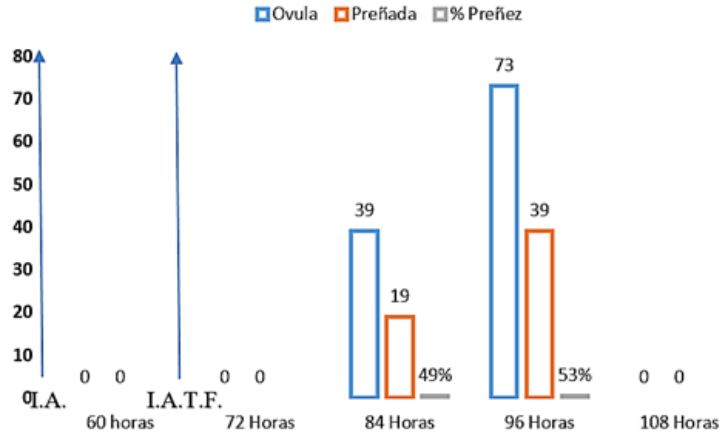
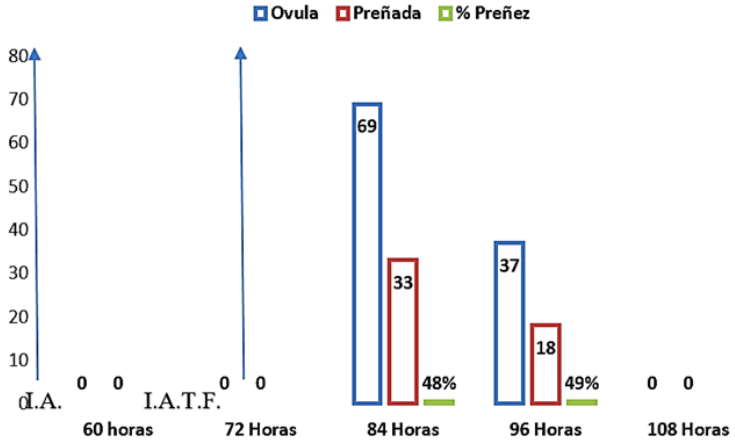


Figure 4. Ovulation response and pregnancy rate with J-Synch treatment without eCG.



3.5 Luteal body size

The luteal body was measured (its diameter in mm) at day 7 post insemination, differing significantly between treatments ($P \leq 0.05$) (T1: 21.6 $\pm$ 0.2b mm; T2: 22.0 $\pm$ 0.2b mm; T3: 22.1 $\pm$ 0.2b mm y T4: 25.0 $\pm$ 0.2a mm). In Table 5 can be observed that variables contribute significantly to the model. It means that the pregnancy percentage is significantly affected by the protocol used, the size of the dominant follicle and the size of the luteal body.

There is 44.5% more chance of getting pregnant with A1 (with eCG 60 hs) compared to T2 (with eCG 72 hs). The conception rate would increase to 22.5% by applying T1 (with eCG 60 hs) compared to T3 (without eCG 60hs). Pregnancy would be 18% higher with T3 (without eCG 60hs) compared to T2 (with eCG 72hs). In all of these cases, the estimated RO confidence intervals include a value of 1. In other words, there might be cases where the chances of pregnancy in both groups are equal, and the difference is caused at random (since a RO=1 means that the chance of getting pregnant is the same for the two categories being compared).

The probability of getting pregnant is four times more in T1 (with eCG 60hs) when compared to T4 (without eCG 72 hs). It is almost three times more likely to get pregnant with T2 (with eCG 72hs) in comparison with T4 (without eCG 72hs). It is 3.5 times more likely on the T3 (without eCG 60hs) when compared to T4 (without eCG 72hs). At eight days it is almost four times likely to pregnant a cow than

the same chance at six days. There is a 50% greater chance of pregnancy when the follicle size increases by 1mm and 35.2% higher when the luteal body size increases by 1mm.

Table 5. Interaction of variables and pregnancy rate.

Effect	DF	Wald's χ^2	Pr > χ^2
Protocol	3	372.609	<.0001
Day	1	298.451	<.0001
TF mm	1	461.369	<.0001
TCL mm	1	739.256	<.0001

4 Discussion

Brown Swiss cows and their crosses with *Bos indicus* were the most used for the production in the Ecuadorian Amazon, although there is a great need to improve reproductive indicators (Moyano et al., 2015). Within a set of problems that surround, among the most important is the delay in the restart of ovarian activity after the delivery (Guáqueta et al., 2014). Currently, there are several protocols available in Fixed Time Artificial Insemination (FTAI) to be used in cows for meat and milk (Colazo et al., 2009; Bó et al., 2018b; Uslenghi et al., 2014), but they need to be revalidated in the Amazon conditions and in Brown Swiss cows and their crosses with *Bos indicus*.

The early results obtained in this work with J-Synch with and without eCG in 448 Brown Swiss cows and their inseminated crosses show that 230 cows were pregnant, representing 51.3%, which agrees with the results reported by Baruselli et al. (2005) and López (2001) in the tropics, and also coincide with those found by López et al. (2014) and Quinteros (2009) who conducted the research in the Ecuadorian Amazon. In addition, the pregnancy percentages are consistent with those obtained by other researchers using similar protocols of prolonged proestrus J-Synch in the Amazon, when eCG was administered and they were inseminated at 60 hours of having removed the device with 61% (70/115) of pregnancy, compared to the inseminated at 72 hours adding eCG, showing 47% (52/111) of pregnancy (López, 2017; Yánez-Avalos et al., 2018).

Table 6. Estimated coefficients and maximum likelihood method

Parameter	DF	Estimate	Standard Error	Wald's χ^2	Pr > χ^2
Intercept	1	-133.388	11.689	1.302.273	<.0001
Protocol	1	14.499	0.2523	330.160	<.0001
Protocol	1	10.820	0.2369	208.638	<.0001
Protocol	1	12.468	0.2417	266.001	<.0001
Day	1	13.733	0.2514	298.451	<.0001
TF mm	1	0.4171	0.0614	461.369	<.0001
TCL mm	1	0.3165	0.0368	739.256	<.0001

All variables are statistically significant in the model (P≤0.001 in all cases).

Artificial insemination programs to detect estrus are affected and have a low efficiency in the tropics because the estrus has a short duration during the day and a tendency to express at night (López, 2014). The expression of estrus was shown in 51% of the total of animals, below 90.4% reported by López (2017) and De La Mata et al. (2015). In turn, these data contrast with those reported by López (2017) who showed that 78.2% of mixed Swiss Brown cows presented estrus when treated with the J-Synch protocol in the Amazon with a free grazing diet. One of the explanations of the low expression percentage of estrus in cows in this work could be that they are individually tied in confined spaces, they cannot express the previous symptoms of the onset of estrus (sexually active group) such as the friction and game between the treated cows, thus they cannot be mounted between them; therefore, the detection of estrus is reduced to subjective observation by the person in charge of other less

expressive symptoms.

The results of the diameters of the ovulatory dominant follicles found in this research agree with those quoted above by López (2017), who showed that the J-Synch protocol had the lowest value of 8.9 ± 0.1 mm, when the device was removed, but it showed larger size of 13.5 ± 0.1 mm, at the time of the FTAI, compared to the treatment Cypriot Cypionate of Estradiol which had a larger size of 9.7 ± 0.1 mm when the device was removed and 12.4 ± 0.1 mm at the time of FTAI. The Benzoate treatment of Estradiol had a size 9.5 ± 0.1 mm when the device was removed and 12.9 ± 0.1 mm to the FTAI, being smaller than the one obtained by the dominant ovulation follicle of the J-Synch treatment.

An experiment in Uruguay with heifers for meat and in another environment, showed that the J-Synch group had an average ovulation schedule of 93.7 ± 12.9 hours (De La Mata et al., 2015). In this work, the average ovulation schedule for J-Synch with eCG at 60 and 72 hours was 94.5 ± 0.9 hours and for J-Synch without eCG at 60 and 72 hours was 88.0 ± 1.1 hours, which agrees with the results found by De La Mata et al. (2015), who by using a J-Synch protocol obtained an ovulation average of 93.7 ± 12.9 hours and those reported by López (2017) who obtained an ovulation interval of 87.7 ± 0.6 hours when using Brown Swiss in the same environment.

The results of the size of the ovulatory dominant follicles found in this work showed that when the progesterone device was removed, there were significant differences ($p \leq 0.05$) that indicated a larger size for *T2* (9.7 ± 0.1 mm); in the same way, the size of the follicle at the time of the artificial insemination expressed significant differences ($p \leq 0.05$) with a larger size for *T4* (13.2 ± 0.1 mm) and at the end of the estrus the size of the follicle showed significant differences ($p \leq 0.05$) with a larger size for *T3* (13.9 ± 0.2 mm). *Lopez2017* reported in the moment of the removal a size 8.9 ± 0.1 mm and 13.5 ± 0.1 mm at the time of artificial insemination, using Brown Swiss mixed cows in the Amazon with a free grazing regimen for J-Synch treatment. Yánez-Avalos et al. (2018), also using Brown Swiss cows in the Amazon found that the follicle size corresponded to J-Synch with eCG 60 hours of 9.53 ± 0.1 mm at the time of removing the progesterone device and to J-Synch with eCG 72 h of 9.87 ± 0.1 mm whereas, at the time of insemination for J-Synch with eCG 60 hours it was 12.3 ± 0.1 mm and for J-Synch with eCG 72 h it was 11.9 ± 0.1 mm, without significant differences. De La Mata et al. (2015), found that the incorporation of eCG into the J-Synch protocol causes a better moment to perform insemination, making the insemination easier in larger groups of animals, where the J-Synch group inseminated at 60 hours had pregnancy percentages of 60.6 % ($P \leq 0.05$) than those in the same group that were inseminated at 72 hours.

Table 7. Maximum Likelihood Estimate Analysis

Odds ratio	Estimate	Confidence limits	
Proto with eCG 60 vs with eCG 72	1.445	0.962	2.169
Proto with eCG 60 vs without eCG 60	1.225	0.805	1.865
Proto with eCG 60 vs without eCG 72	4.263	2.600	6.990
Proto with eCG 72 vs without eCG 60	0.848	0.562	1.279
Proto with eCG 72 vs without eCG 72	2.950	1.855	4.694
Proto without eCG 60 vs without eCG 72	3.479	2.166	5.588
day 6 vs 8	3.949	2.412	6.463
TFmm	1.518	1.345	1.712
TCLmm	1.372	1.277	1.475

Núñez-Olivera et al. (2014) assessed the effect on the prolongation of the proestrus and how it affected the development of the ovulator follicle when a dose of eCG, and GnRH was applied to them at 48 and 72 hours after the device was removed, showing that at 48 hours the diameter was 8.8 ± 0.4

mm and the FTAI of 12.3 ± 0.5 mm; at 72 hours of having removed the device, the diameter was 8.2 ± 0.3 mm and the FTAI was 13.5 ± 0.3 mm without significant differences.

Van-Eerdenburg et al. (2002) found that cows with larger follicle size had a higher chance of ovulation. In accordance with the above, Yánez et al. (2016) found that cows with a larger follicular size at the time of artificial insemination were fourteen times more likely to get pregnant (pregnant: 13.4 ± 1.1 mm; empty: 10.4 ± 1.3 mm).

In the work, significant differences ($p \leq 0.05$) were found in the size of the luteal body on day 7 post insemination between the treatments used. De La Mata et al. (2015) found that the size of the luteal body between days 4 and 13 after ovulation tended to be higher ($P \leq 0.074$) in the J-Synch group compared to the conventional protocol. The size of the luteal body would be related to the higher or lower serum concentrations of progesterone that would affect fertility (Vasconcelos et al., 2001; Busch et al., 2008; Mann, 2009).

Previous cited work indicated that the incorporation of the J-Synch protocol in extreme environmental conditions allowed to improve the rates of pregnancies in cows with breeding, with anestrus and with extensive open period, in double purpose cattle and for meat in tropical climates. However, it is necessary to continue to add a larger number of Brown Swiss cows and their crosses, which are the most frequently used cow in production systems in the Amazon, where the J-Synch protocol is used to consolidate the results.

5 Conclusions

J-Synch treatments plus eCG at 60 hours and 72 hours had different behavior in the follicular development and the size of the Luteal Body. The percentage of pregnancies did not differ between the treatments. Although, there was a 44.5% more chance of getting pregnant with T1 compared to T2. In addition, pregnancy would increase by 22.5% by applying T1 compared to T3, and pregnancy would be 18% higher with T3 when compared to T2.

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