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EFFECT OF THE ADDITION OF eCG ON DAY 14 POST-FTAI ON THE PREGNANCY RATE IN COWS FROM THE ECUADORIAN AMAZON

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ABSTRACT: *The effect of the addition of eCG on day 14 after FTAI was evaluated in 200 cows from the Ecuadorian Amazon, with a calf at the foot, of Brown Swiss breeds and their crosses (Bos indicus). Two treatments (T): T1 (J-Synch + eCG day 6) and T2 (J-Synch + eCG day 14 post-FTAI). The pregnancy rate for T1 (57%) and T2 (48%) without showing a significant difference (p≥0.05). The pregnancy percentage was higher (62.5%) in cows in estrus (125/200). Plasma progesterone levels differ between T1 and T2, evaluated on day 14: (7.1 ng/mL); (13.5 ng/mL), respectively, as well as on day 30: T1 (9.6 ng/mL) and T2 (13.7 ng/mL), (p≤0.05). The diameter of CL measured on day 14 (20.8 ± 3.3 mm) and day 30 (28.2 ± 6.2 mm) differed significantly (p≤0.05). Estrous expression is positively associated with an increased pregnancy rate, but improving eCG on day 14 post-FTAI does not improve the pregnancy rate in Ecuadorian Amazon cows.*

KEYWORDS: eCG, Estrus, J-Synch, Amazon.

INTRODUCTION

Equine chorionic gonadotropin (eCG) has generated a lot of expectations on its timing and dosage. This hormone is a high molecular weight glycoprotein produced by the endometrial cups in the pregnant mare between 35 and 100 days (Murphy & Martinuk, 1991). Due to its high molecular weight and the presence of sialic acid, the eCG molecule is negatively charged, which hinders its glomerular filtration and further increases its half-life. Due to all these factors, the half-life of eCG when applied in cattle is long (Souza et al., 2008). This hormone expresses a biological action of FSH and LH in a 1.4:1 ratio (Steward et al., 1976). It has been shown that treatment with the addition of eCG increases the development of the dominant follicle and produces the ovulation of a larger follicle resulting in a larger and more functional CL, acting on embryo survival (Baruselli et al., 2004).

When eCG is administered in anestrus cows, it creates conditions to stimulate follicular growth and ovulation, even in cows with depressed gonadotropin release. Its use has shown a positive effect in herds with low cyclicity rate (anestrus), in recently calved animals (postpartum period less than two months), in animals with compromised body condition (≤ 2.5 on a scale of 1 to 5) (Mapletoft et al., 2022), and in animals with compromised growth of the dominant follicle (Marques et al., 2005). Due to its FSH and LH action and long half-life, eCG has been used in embryo recipient synchronization protocols (Baruselli et al., 2000). The use of 400 IU of eCG at the time of intravaginal device removal resulted in increased blood plasma progesterone concentration and pregnancy rates in cows with calves at foot treated during postpartum anestrus (Baruselli et al., 2004; Bó et al., 2007).

Research conducted in Ecuador with conventional protocols, pregnancy rate, according to Ortiz (2017), considers that the addition of hormones such as Equine Chorionic Gonadotropin (eCG) can provide important data in studies to improve the pregnancy rate in cows in the province of Pastaza. In recent times, the protocol called J-Synch has undergone some variations, without altering its initial form, such as a dose of intramuscular (IM) Estradiol Benzoate (BE) and the insertion of a progesterone (P4) intravaginal device (DIB) to synchronize the onset of a new follicular wave with a reduced period of DIB insertion with p4 of 6 days instead of 7 or 8 days, applying a GnRH as ovulation inducer at 72 h after DIB removal together with IATF guaranteeing prolonged proestrus (de la Mata et al., 2012). In addition the administration of 400 IU of equine chorionic gonadotropin (eCG) upon removal of the device can induce follicle growth and generate ovulation, to subsequently perform IATF (Mayorga et al., 2020).

While it is true that eCG stimulates follicle development in dominance, it is also associated with an increase in the corpus luteum (CL) diameter by stimulating the synthesis of progesterone (P4) (De Carvalho et al., 2016). Bó et al. (2011) describe a favorable use of eCG applied after artificial insemination concerning embryonic losses due to its characteristics of a long half-life time possesses an effect equivalent to Follicle Stimulating Hormone (FSH), and Luteinizing Hormone (LH) in the follicle, managing to favor the growth and maturation of follicles and ovulation (Vera, 2017). Thus it can also bind to the LH receptors of the CL, reaching an increase of luteal cells, which are responsible for the synthesis of about 80% of progesterone (P4), providing more volume and more capacity for P4 production (Mello et al., 2014). P4 is fundamental for embryonic survival and development, as well as the identification of pregnancy, associating this with an increase in the rate of embryo development (Butler et al., 2016). Thus, the present work seeks to evaluate the effect of eCG application at day 14

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post-IATF and its response on pregnancy percentage, follicular development, and P4 concentrations in dual-purpose cows in the Ecuadorian. Amazon.

METHODOLOGY/MATERIALS AND METHODS

The work was carried out from June to December 2022 in the province of Pastaza - Ecuador, which has an average temperature of 25°C to 30 °C, a relative humidity of 80 - 90%, an average rainfall of 4000 - 5000 mm/year and an altitude that varies from 600 to 900 meters above sea level (GADMP, 2019). We used 200 cows all in production with calf at foot, of Brown Swiss breeds and their multiparous crosses, with a body condition (CC) ≤ 2.5 (scale from 1 to 5) (Edmonson & Lean, 1989), aged 34-60 months, weighing >350kg and 90 to 100 days open on average and grazing in natural fields.

Treatment 1 (n=100) J-Synch + eCG protocol (day 6). Day zero, 2 mg BE plus DIB 0.5g P4. On day six, the device was removed and 500 µg of PGF2α (Cloprostenol) plus administration of 400 IU of eCG was applied. Ultrasonography was used to measure the diameter of the largest follicle at the onset of proestrus; a paint marker was placed at the base of the tail to detect the presence of estrus prior to FTAI. Cows that were in estrus were inseminated at 60 h and those that were not in estrus were inseminated at 72 h plus the administration of GnRH (gonadorelin acetate) at the time of FTAI. On day 9, the measurement of the diameter of the largest follicle continued. On days 14 and 30 post FTAI, the corpus luteum (CL) diameter was measured and a blood sample was taken for progesterone analysis.

Treatment 2 (n=100) J-Synch + eCG protocol (day 14 post-FTAI). Day zero, 2 mg BE plus DIB 0.5g P4. From this group on day six, the device was removed and 500 µg of PGF2α (Cloprostenol) was applied. Ultrasonography was used to measure the diameter of the largest follicle at the onset of proestrus, and a paint marker was placed at the base of the tail to detect the presence of estrus before FTAI. The cows that were in heat were inseminated at 60 h and those that were not in heat were inseminated at 72 h plus the administration of GnRH (gonadorelin acetate) at the time of FTAI. On day 9, the measurement of the diameter of the largest follicle was continued. On day 14, 400 IU of eCG was administered and on day 30 post-FTAI, corpus luteum size (CL) was measured and a blood sample was taken for progesterone analysis.

For both treatments, gestation diagnosis was performed 40 days after the FTAI by ultrasonography (Minitube ultrasound scanner with a 5 Mhz linear probe).

For the analysis of serum P4 concentrations, 20 samples were analyzed for this essay: T1 = 10 samples; and T2 = 10 samples, analyzed individually in duplicate using Progesterone Kits (iChroma™Progesterone CFPC - 21- Korea). Fluorescence Immunoassay (FIA) technique was used for the quantitative determination of P4 in serum. The sensitivity of the minimum detectable concentration of progesterone is distinguished in a working range: 0.127 to 127.2nmol/L and 0.4-40ng/mL with a confidence limit of 99%. The intra-assay coefficient of variation (CV) was low (5.2%) and high (6.2%) (Boditech, 2020).

For the two treatments, each variable was estimated as arithmetic mean (X) and standard error. Significant differences between treatments were tested by applying an analysis of variance (ANOVA) to a ranking criterion and Tukey-Kramer HSD multiple comparisons tests ($P \leq 0.05$)

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of binary data (0 empty and 1 pregnant) to determine the influence of the different variables and their interactions on pregnancy rate. An alpha 0.05 was used to determine significant differences and 0.10 as a trend value. Statistical analyses were performed using JMP software (JMP®, 2003) version 5.0 for Windows.

RESULTS

Pregnancy percentage: Of the 200 cows used, 105/200 (52.5%) were pregnant and 95/200 (47.5%) cows were left empty. The pregnancy percentage of the protocols was T1 (57%) and T2 (48%) with no significant differences ($p \geq 0.05$) (Table 1).

Table 1: Percentage of pregnancy in T1 and T2, in dual-purpose cows from the Ecuadorian Amazon

Treatments	Nº animals	Pregnant cows	% pregnancy
T1	100	(57/100)	57
T2	100	(48/100)	48
Total	200	(105/200)	52.5

The percentages do not differ ($p \geq 0.05$).

Expression of estrus: There was a 62.5% (125/200) presence of estrus in total animals for both treatments. The percentage of pregnancy in cows with the presence of estrus was 58.4% (73/125) and without the presence of estrus was 42.7% (32/75), showing a significant difference ($p \leq 0.05$) (Table 2).

Table 2: Pregnancy rate in relation to the presence of estrus in the two treatments

Treatments	Nº- animals	with estrus	No estrus	Accumulated pregnancy rate
T1	100	41/66 (62.1%)	16/34 (47%)	57/100(57%)
T2	100	32/59 (54.2%)	16/41 (39%)	48/100 (48%)
TOTAL	200	73/125 (58.4%)	32/75 (42.7%)	105/200 (56.1%)

The percentages differ ($p \leq 0.05$)

Follicular development: The average size (mm) of the follicle diameter obtained on the day the DIB intravaginal device was removed for the T1 synchronization treatment, was 9.46 ± 1.22 mm; while for the T2 treatment it was 9.7 ± 1.72 mm. Values that did not show significant differences ($p \geq 0.05$) (Table 3).

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Table 3: Follicle size upon removal of the DIB device and at the time of the FTAI in two treatments.

Treatments	Follicle removal / mm	Follicle FTAI / mm	% pregnancy
T1	9.5±1.2	12.5±3.0	57
T2	9.7±1.7	12.4±3.7	48

The measurements do not differ (p≥0.05).

Corpus luteum size, progesterone levels, and pregnancy percentage: The average size of the corpus luteum obtained at day 14 post-FTAI for T1 was 20.8 ± 3.3 mm and for T2 was 21.1 ± 3.0 mm. These results are shown in Table 4 without significant difference (p≥0.05). Not being so, the average size of the corpus luteum at day 30 post-FTAI for T1 was 28.2 ± 6.2mm and for T2 was 21.7 ± 4.0 mm showing a significant difference between the evaluated protocols (p≤0.05). The average progesterone value found at day 14 post-FTAI for T1 was 7.06 ng/mL; while for T2 treatment, it was 13.5 ng/mL; just as the progesterone concentration at day 30 post-IATF for T1 was 9.76 ng/mL; while in T2, 13.7 ng/mL was obtained (p≤0.05). But no significant difference was evidenced between P4 on day 14 and day 30, (p≥0.05).

Table 4 shows CL diameter, pregnancy percentage, and serum P4 concentrations at both day 14 and 30 post-FTAI in the two treatments showing no significant difference (p≥0.05).

Table 4: CL size, pregnancy rate and serum P4 concentrations at day 14 and 30 post FTAI subjected to a prolonged proestrus protocol

Treatments	N°	CL (mm) day 14	CL (mm) day 30	P4 (ng/mL) day 14	P4 (ng/mL) day 30	Pregnancy rate (%)
T1	10	20.8 ± 3.3	28.2 ± 6.2	7.1	9.6	60 (6/10)
T2	10	21.1 ± 3.0	21.7± 4.0	13.5	13.7	40 (4/10)
TOTAL	20	21.0 ± 3.0	25.0± 15.9	10.3	11.7	50 (10/20)

The measurements do not differ (p≥0.05).

DISCUSSION

The results obtained in this research allowed evaluation of the use of a dose of eCG at day 14 post-FTAI in dual-purpose cows in the Ecuadorian Amazon, obtaining a pregnancy percentage without significant differences between treatments (Table 1) and showing that the inclusion of eCG did not improve the pregnancy percentage. This could be explained by the ovarian status of the cows before starting the treatments, which Baruselli et al. (2004) describe in their study that eCG will not have a follicular stimulation and ovulation effect in cows cycling or in good body condition. Núñez et al. (2010) did obtain a higher pregnancy rate by administering eCG in cows in anestrus, ovulating, and subsequently expressing a higher concentration of progesterone, which generated a better uterine environment and therefore a better pregnancy rate. Also, to the results reported by Cutaia et al. (2009), using 400 IU of eCG 14 days after

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FTAI in primiparous beef cows with calf at foot and with fair to poor body condition, increasing pregnancy rates (with eCG: 47.2% versus; no eCG: 30.8%).

The pregnancy percentage was higher in cows with the presence of estrus in both treatments ($p \leq 0.05$) (Table 2), similar to that obtained by Ortega et al. (2020), where the pregnancy rate with the presence of estrus was 54.3% and without estrus was 44.4% for the J-Synch treatments plus eCG administration. Animals that show estrus before being inseminated result in higher pregnancy rates due to higher pre-ovulatory serum estradiol concentration, larger follicular diameter, and higher ovulatory rate and therefore developed a CL with higher progesterone secretory capacity during the luteal phase (Sá Filho et al., 2011).

The follicular development obtained on the day the DIB intravaginal device was removed showed no significant differences in T2. These values are numerically superior to those obtained by Yánez et al. (2021), with J-Synch + eCG protocols in the Ecuadorian Amazon, reporting values of 8.9 ± 0.1 mm in follicle size at the removal of the intravaginal device. Like de la Mata et al. (2018) in their J Synch protocol in beef cattle found measurements of 8.3 ± 1.2 mm. Not presenting differences in follicle sizes at the withdrawal of the intravaginal device, Bruschi (2020) determines that a device with progesterone and a dose of estradiol benzoate causes atresia of the follicles for subsequently the appearance of a new follicular wave, which produced a similar follicular emergence in the two treatments.

On the one hand, the average follicle diameter measured on the day of FTAI showed no differences for the treatments (Table 3), finding normal values that agree with Bruschi (2020), when mentioning that a feasible follicle for ovulation should be >10mm. The effect indicated by Bó et al. (2016), when eCG is used at the withdrawal of the P4 device is efficient to stimulate the development of the dominant follicle increasing pregnancy in cows with postpartum anestrus or low body condition. However, the results obtained, without the application of eCG on day 6 (Table 3), comes to be similar case to that of de la Mata (2016) who applied a J-Synch without eCG in his experiment I with an average follicular size at FTAI of 13.0 ± 1.04 mm. It also occurred with Lopez (2017) who obtained a follicular size of 13.5 ± 0.1 mm in a J Synch protocol without using eCG at device withdrawal in the Ecuadorian Amazon. These results obey a physiological pattern of follicular growth, as mentioned by Dorneles et al. (2013) who indicate that follicles ovulate when presenting an approximate size of 10 or 12 mm, and even Andringa et al. (2013) found a maximum preovulatory follicle size of 16.2 ± 1.7 mm under normal conditions without previous hormonal management.

The size of the corpus luteum found in both protocols (Table 4) in this work was similar to that found by Pilla et al. (2022) at day 14 (21.6 ± 5.1 mm) in their J-Synch protocol plus the application of eCG at day 6 in dual purpose cows from the Ecuadorian Amazon, without presenting significant differences, when compared to a conventional protocol. Ortiz (2017), who measured the diameter of CL in crossbred cows in the province of Pastaza in earlier periods (day 7 post insemination), reported statistically similar values between treatments: with eCG at withdrawal and with eCG at day 14 post-FTAI, with sizes of 20.99 ± 0.91mm and 19.23 ± 0.82 mm respectively. The average size of the corpus luteum at day 30 post-IATF for T1 was superior to T2 showing a significant difference ($p \leq 0.05$) (Table 4). These values coincide with that described by Núñez (2014) where performing CL measurements from day 14 to 22. His study found out that it was higher in cows that received eCG at withdrawal concerning those that did not. In this way, Kojima (2003) mentions that the middle phase of corpus luteum development happens in the first week after ovulation, in which it undergoes rapid

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morphological changes. Therefore, in our study, the growth of the CL could be influenced by the increase in the number and function of luteal cells and other types of cells (Niswender et al., 2000), which occur hours after ovulation and which would be responsible for the final size of the CL and which would later guarantee adequate P4 synthesis and thus the maintenance of gestation.

The average progesterone value found in this study showed significant differences between both treatments (Table 4). The results are higher than those presented by Gamboa (2020), where at 11 days post ovulation, he found 7.65 ng/mL in his J-Synch protocol. Also, it is superior to those reported by Pilla et al. (2022) in conditions of the Ecuadorian Amazon showed a P4 value for day 14 post-IATF of 6.72 ng/mL.

Similarly, Busch et al. (2008) in their research indicate that the corpus luteum with its size and progesterone synthesis has an accelerated increase until day 8 of the estrous cycle, being the size of the corpus luteum determinant for progesterone synthesis. However, it is important to note that in this research, on day 14 post-FTAI, the cows studied, although the size of the corpus luteum was statistically similar in the two treatments, the concentration of P4 was different. Progesterone concentration at day 30 post-FTAI showed significant differences between treatments (Table 4). This increase is similar to that obtained by Núñez (2014), where serum progesterone concentrations from day 14 to 22 observed an increase in progesterone concentration in pregnant cows, the other hand, evidenced by an average concentration of 11.0 ± 0.6 ng/mL of P4 between days 15 to 18 in cows that administered eCG at day 14 post-FTAI to those that did not receive eCG. De la Mata (2016) indicates that these changes in P4 concentration in J-Synch protocols improve the condition of the endometrial environment of the uterus, associating this to P4 exerting an effect on the endometrium by stimulating embryo development and elongation. Spencer et al. (2006) mention that there is no certain optimal value to which progesterone levels should reach; moreover, Diskin et al. (2016) mentions that a high concentration of P4 affects fertility, which can be attributed to embryonic losses due to inadequate P4 values.

CONCLUSIÓN

The treatments evaluated show that estrous expression is positively associated with higher pregnancy rates, but the addition of eCG at day 14 post-FTAI, despite having high concentrations of progesterone, did not improve the pregnancy rate in dual-purpose cows from the Ecuadorian Amazon.

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