Chapter 10

# Antiluteolytic Strategy for Bovine Embryo Transfer Programmes

Néstor Isaías Tovío Luna, Arturo Duica Amaya and Henry Alberto Grajales Lombana

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60425

**Abstract**

This paper presents a comprehensive review of the problematic issue of embryonic mortality in cattle and develops possible strategies towards a hormonal antiluteolitic. A recent and extensive investigation using eCG is also described.

The paper evaluates the effect of the application of 400 IU of equine Chorionic Gonadotrophin (eCG) on day 5 or 8 of a synchronization protocol for embryo transfer upon follicular development (day 9), luteal development (day 17), progesterone concentration "$P_4$" (9 and 17) and percentage of pregnancy (day 52) in 70 Holstein heifers. The relationships between dominant follicle diameter (DFD) (day 9) and the luteal volume (VL) (day 17), as well as the concentration of P4 (day 17) are analysed without eCG treatments; the DFD (day 9), VL (day 17) and concentration of $P_4$ (day 17) versus percentage of pregnancy (day 52) are then analysed with the treatment. There was no effect ($P > 0.05$) of the day of eCG administration (5 or 8) on the number of dominant follicles, but $P < 0.05$ for diameter. The day of eCG administration (5 or 8) had no effect ($P > 0.05$) on the number of corpora lutea, VL, $P_4$ concentration (9 and 17) or pregnancy (day 52). There was no relationship ($P > 0.05$) between the DFD (day 9) and VL, but $P < 0.05$ between the VL and $P_4$ levels (day 17). In analysing the relationship between treatments (eCG day 5 or 8), the DFD (day 9), VL (day 17) and the concentration of $P_4$ (day 17) versus percentage of pregnancy (day 52), we observed that the only positive correlation ($P < 0.1$) existed between this variable and the concentration of $P_4$.

**Keywords:** eCG, Corpus luteum, Dominant Follicles, Progesterone, Embryo Transfer

## 1. Introduction

About 25-40% of embryonic losses are detected during the first days of pregnancy in female recipients of bovine embryos [1]. It is observed that most of these females return to heat on the expected date after 20-22 days, showing a whole and normal sexual cycle (oestrus cow repeaters) [2]; it is therefore suggested that embryo mortality (EM) might arise between days 7 and 17, the period from embryo transfer (ET) to the maternal recognition of pregnancy (MRP) [3]. As for pregnancy losses occurring between days 28 and 98 (at the occurrence of MRP), percentages ranging between 7% and 33% have been calculated [4].

It has been suggested that during pregnancy establishment there is a well defined "critical period" from day 15 to day 17 [3]; we can suppose that reproductive biology during this period would be multifactorial and complex, where the endometrium receives a non-suitable antiluteolytic signal, of course without blocking prostaglandin $F_{2\alpha}$ ($PGF_2\alpha$) endometrial production, triggering the lysis of the corpus luteum (CL) (maintaining pregnancy depends upon CL functionality) [1]. At that point, antiluteolytic signal is generated by the embryo into mononuclear trophoblast cells, which secrete interferon *tau* (IFN-τ), thereby blocking $PGF_2\alpha$ synthesis produced at endometrial level [5]. The latter process suggests that embryo loss could occur because of a weak or inadequate signal due to an asynchrony between a decreasing progesterone ($P_4$) and the degree of embryo development, so it does not inhibit the synthesis of uterine $PGF_2\alpha$ [6].

The critical nature of the period of recognition, apposition and adhesion of the embryo to uterine endometrium during implantation demands strict synchronization between the transferred embryo and the recipient, emphasizing the importance of both the uterine environment and the embryo signals generating MRP [7] [8]. These signals must be released at the time and concentration required to guarantee CL structure and function maintenance, generating continuous $P_4$ production for embryotrophic environment maintenance to support the normal development of the conceptus (the embryo including all the linked layers) [2].

In relation to the influence of $P_4$ on certain events related to pregnancy maintenance from early stages and the ability of $PGF_2\alpha$ to instigate luteolysis, a number of hormonal strategies to maintain pregnancy have been proposed and developed [9] [10]. These tend to be based on making the $P_4$ secretion capacity of the CL more effective: the secretion should be timely, thus ensuring a proper uterine environment for development of the embryo transplanted to the recipient bovine female. All of this is aimed at increasing the pregnancy rate in ET programmes [2].

http://dx.doi.org/10.5772/60425

According to some reports, the higher the plasma $P_4$ concentration, the better the uterine environment for developing conceptus [11]. It should be noted in this context that any variation of $P_4$ concentration is crucial to modulate expression and secretion of growth factors, cytokines and proteins that affect the uterine environment for endometrial receptivity and embryo viability processes [12].

Consequently, it has been proposed that by providing direct or indirect $P_4$ sources to females during the first days of pregnancy, thus improving the uterine environment to enable the conceptus to develop appropriately, the percentage of embryo loss will be decreased. This leads to better synthesis and more timely secretion of IFN-τ, as long as this secretion is influenced by the embryo development status [13].

## 2. Use of equine Chorionic Gonadotropin (eCG) in bovine embryo transfer programmes

The relationship between pregnancy rate and plasma $P_4$ concentration, according to the CL size in recipients of bovine embryos, has been much debated in the literature. Some researchers have verified a positive correlation between these variables, establishing that the greater the CL area, the greater the plasma $P_4$ concentration, and, subsequently, the higher the pregnancy rate [10] [14]. Other reports, however, have not been able to observe this relationship and effect [15].

Similarly, plasma $P_4$ concentration during dioestrus has been positively correlated to an embryo's ability to secrete IFN-τ, thus triggering increased pregnancy rates [16] [17]. This might suggest that increased $P_4$ concentration during the "critical period" improves the uterine environment for a developing embryo, generating an effective MRP process as it stimulates the secretion of IFN-τ and antiluteolytic agents at the right time by trophoblast cells [18].

One can thus see $P_4$ as a forerunner of the various components making up the embryotrophic environment; therefore, any change in its concentration is critical to modulate expression and secretion of growth factors, cytokines and proteins that affect the uterine environment for endometrial receptivity and embryo viability processes [19]. Accordingly, we may assume that by providing direct or indirect $P_4$ sources to females during the first days of pregnancy, percentage of embryo loss may be decreased due to having an improved uterine environment where the embryo might follow a proper development process (blastocyst stage) with synchronized synthesis and secretion of IFN-τ. This latter is undoubtedly influenced by the status of an embryo's development [13].

In order to increase pregnancy rates, hormone treatments have been used in females included in programmes of fixed-time artificial insemination (TAI) and fixed-time embryo transfer (FTET) using equine chorionic gonadotropin (eCG) [10], [20].

eCG, formerly known as pregnant-mare serum gonadotropin (PMSG) is a glycoprotein hormone with a molecular weight of 45 kDa. It has a three-day span life and is produced by

endometrial calyces in pregnant mares from day 40 to day 130 [21]. The hormone is composed of two subunits, $\alpha$- and $\beta$. Subunit $\alpha$ is encoded by a gene common to any glycoprotein; the gene coding for subunit $\beta$ gives the hormone specificity [22].

This hormone binds to FSH and LH follicle recipients as well as LH recipients of CL, thereby creating conditions for follicle growth, ovulation and luteinization [20]. The prevalent action is given by FSH, leading to a formation of accessory corpora lutea, typical for a pregnant mare [23].

eCG application at the expected time of a new wave of follicle growth has been shown to lead to excellent ovulation efficiency (per dosage) and development of a larger-diameter dominant follicle, thus determining a greater number of corpora lutea or a larger corpus luteum [10]. Higher plasma $P_4$ concentrations and a higher rate of conception and pregnancy are seen in comparison to treatments without application of this hormone, both in *Bos taurus* and *Bos indicus* cattle and their crossbreed [24] [11]. However, other researchers have reported no difference between plasma $P_4$ concentration and the number of corpora lutea in pregnant females; in fact, we established higher pregnancy losses in females with double ovulation, suggesting that too much plasma $P_4$ could alter the hormonal uterine balance, damaging the embryotrophic environment for a developing embryo [25].

It has been verified that with eCG application on day 8 (as dominant follicle established), within a synchronization protocol of bovine embryos in recipient females (*Bos indicus*/*Bos taurus*), one-time larger corpora lutea are achieved in comparison to application of the same hormone on day 5 (as growth of a new follicle wave begins), but no differences have been determined between the treatments in the produced plasma $P_4$ concentration or pregnancy rates [26]. Related reports have confirmed that rate of use (transferred females/synchronized females*100) improves when eCG application occurs on day 8 within a fixed-time embryo transplant protocol with *Bos indicus* recipients [27].

For eCG application on day 8 of synchronization, double ovulation of only 2% has been reported in embryo recipients, but it is evident that application of this hormone can achieve one-time larger corpus lutea, thus increasing the pregnancy rate [13]. Similarly, Quezada and Ortiz [27] established that eCG application on day 8 improves the utilization rate; however, they were unable to establish that the hormone enhances the CL area.

Other hormone strategies have been used in several studies to increase plasma $P_4$: cows and buffaloes have been used for treatments with Gonadotropin-Releasing Hormone (GnRH), Luteinizing Hormone (LH), human Chorionic Gonadotropin (hCG), and $P_4$ slow-release devices [28].

hCG (produced in the trophoblastic syncytium of pregnant women) has been applied to synchronization treatments on day 6, resulting in higher pregnancy rates compared to groups without application of this hormone [29]. These results also suggest that hCG, which has LH action, induces ovulation and formation of accessory corpus luteum (co-dominance), which increases the plasma $P_4$ concentration and pregnancy rate in recipient females of bovine embryos [2].

http://dx.doi.org/10.5772/60425

In addition, eCG has an advantage over other gonadotropins used to support hormone cattle in its stimulation by carbohydrates, especially sialic acid, in relation to subunit alpha, which gives a higher lifespan (see Table 1). This feature, allied to the hormone's molecular weight, makes glomerular filtration harder, thus increasing its lifespan even more [30].

| Hormone | Molecular Weight ng/ml | Carbohydrates (%) | Sialic Acid (%) | Span Life |
|---|---|---|---|---|
| Luteinizing (LH) | 28000 - 34000 | 12 - 24 | 1 - 2 | 30 minutes |
| Stimulating Follicle (FSH) | 32000 - 37000 | 25 | 5 | 2 hours |
| Human Chorionic Gonadotropin (hCG) | 38000 | 32 | 8 -5 | 11 hours |
| Equine Chorionic Gonadotropin (eCG) | 68000 | 48 | 10.4 | 26 hours |

**Table 1.** Features of gonadotropic hormones. Adapted from Knobil and Neill, 2006.

### 2.1. Effect of eCG upon pregnancy rate among recipient heifers of bovine embryos

A study was proposed and developed in order to assess the effect of applying a treatment of equine Chorionic Gonadotropin (eCG) on day 5 or day 8 within a synchronization protocol for a fixed-time embryo transfer (FTET) upon development (number and mm diameter) of dominant follicles on day 9, luteal development (number and volume in $mm^3$) on day 17, plasma $P_4$ concentration (ng/ml) on days 9 and 17, and pregnancy rate (%) on day 52, using 70 Holstein heifers (eCG day 5, n = 42 and eCG day 8, n = 28) as embryo recipients in high tropical areas in Colombia.

To meet the research goal two treatments were proposed in control protocols for the oestrus cycle, where only the eCG application day was changed (day 5 or 8) (Figures 1 & 2).

Regardless of synchronization treatment (eCG day 5 and eCG day 8), we determined $P_4$ profiles for three samples (days 5, 9 and 17) for every heifer in the analysis (n=70). A relationship between volume of corpus luteum and plasma $P_4$ concentration on day 17 (sample 3) was established.

By a logistic function the treatment effects (eCG days 5 and 8) upon follicle diameter (dominant follicle – day 9), total luteal volume (day 17), and $P_4$ concentration (day 17) were determined upon pregnancy diagnosis (day 52).

#### *2.1.1. Number of dominant follicles (day 9)*

This analysis shows that every female used for both treatment 1 (eCG day 5) and treatment 2 (eCG day 8) had at least dominant follicle as a variable with no evident differences ($P>0.05$) (see Table 2).

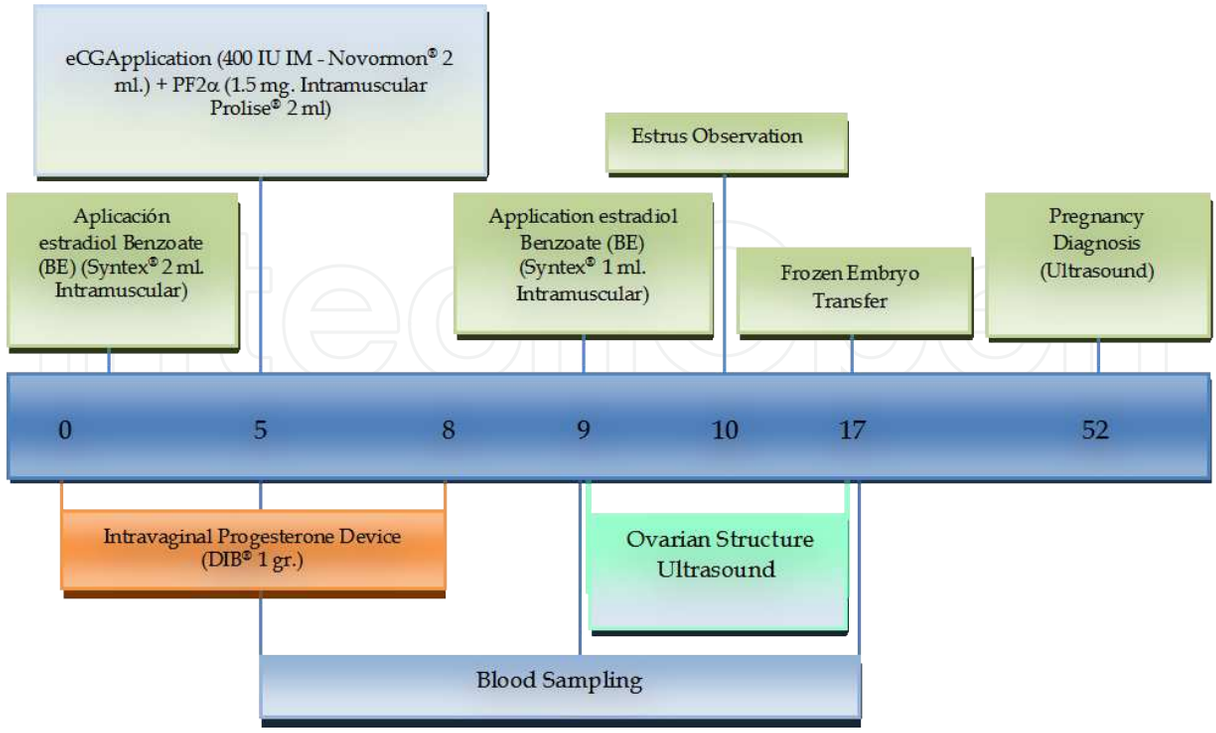


**Figure 1.** Treatment 1: Synchronization protocol of oestrus cycle using eCG application on day 5 (in relation today 1 protocol).

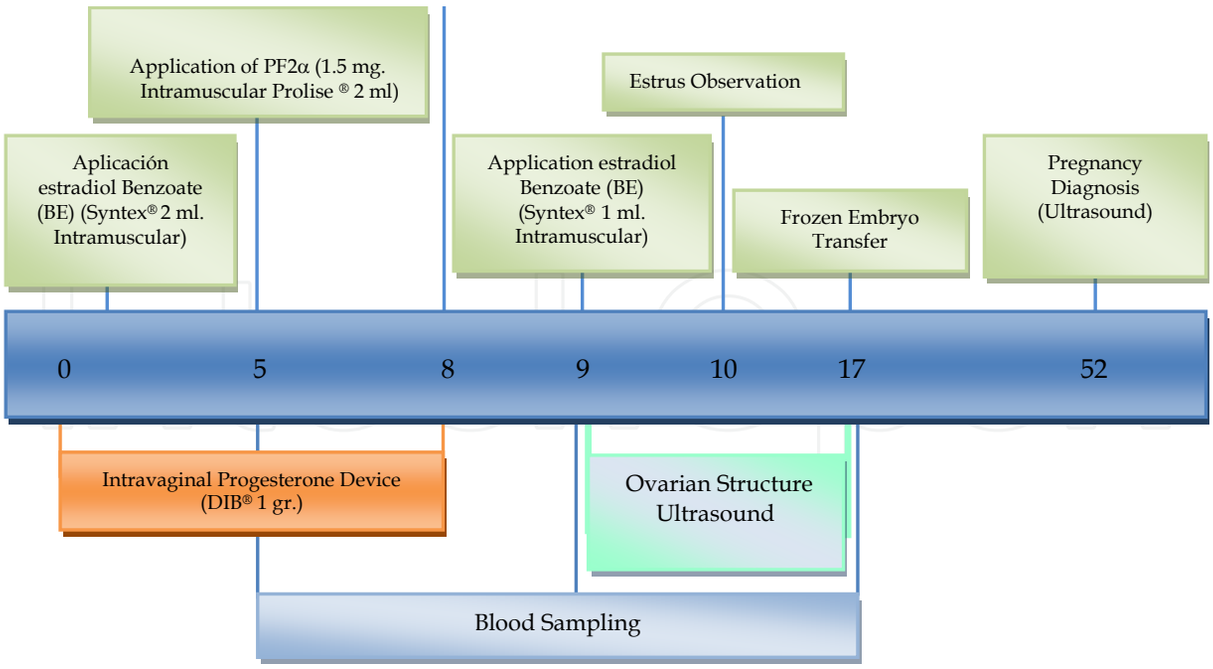


**Figure 2.** Treatment 2: Synchronization protocol of oestrus cycle using eCG application on day 8 (in relation to protocol day 1).

http://dx.doi.org/10.5772/60425

| Treatment | Dominant Follicle (Number) day 9 | | | Diameter (mm) Dominant Follicle day 9 | | |
|---|---|---|---|---|---|---|
| | Min. | Max. | Avrg.[1] | Min. | Max. | Avrg.[1] |
| eCG day 5 (n=42) | 1 | 2 | 1.1 ± 0.3 a | 10 | 19 | 11.7 ± 054 a |
| eCG day 8 (n=28) | 1 | 1 | 1 ± 0 a | 9.2 | 16 | 10.1 ± 0.6 b |
| Total Sample (n=70) | 1 | 2.0 | 1.1 ± 0.2 | 9.2 | 19 | 10.9 ± 0.41 |

**Table 2.** Dominant follicles on sampling day 9. [1]Results of follicle diameter are expressed as an average (mean) ± Standard error of the mean (SEM) while follicle values are expressed as an average ± Standard Deviation (SD).

This result differs from previous studies where addition of eCG on day 5 in a synchronization protocol for the oestrus cycle caused a reflection cascade at molecular level, triggering development of more than one dominant follicle [31]. Since these control oestrus cycle treatments, where a follicle wave growth using $P_4$-and-oestradiol implant is synchronized (as in this work), the emergence of a new follicle wave occurs after four to five days, an effect perhaps caused by blood hormone levels required to provoke a negative feedback. This affects the synthesis and secretion of gonadotropin-releasing hormone (LH) and alters the normal follicle development, which suggests a leading regression of growing follicles (FSH-dependent) and hinders progress of the phenomenon of dominant follicle ovulation (LH-dependent), generating a regression and starting a new follicle wave [32].

When eCG is applied on day 5, the follicle wave is at a very early stage of development; consequently, the eCG could lead to stimulation in recruitment and selection of more than one dominant follicle with increasing mRNA synthesis coded for gonadotropic hormone recipients (FSH and LH) at theca-and-granulosa cell level [10]. This effect in this study was only observed in four of 42 heifers (9.5%).

In relation to the above, Nasser et al. [26] applied 400 IU of eCG to two groups of Nellore/Angus -crossbreed recipient heifers (eCG day 5 versus eCG day 8) of 22-30 months in age; this was supplemented with 2 kg/day concentrate, establishing differences ($P<0.05$) according to the number of dominant follicles on day 8, with a higher number of follicles applied on day 5 (start of a new wave of follicle growth), thus demonstrating the follicle-stimulating action of eCG. The hormone stimulates the mRNA synthesis encoded for FSH follicle recipients in developing follicles [33]; therefore, more than one follicle is chosen because there are no dominant follicles at that time (day 5 of application in relation to the start of the control protocol of the oestrus cycle) that could inhibit other developing follicles. For eCG applied on day 8 (follicle dominance established) only one dominant follicle stimulation (eCG linking FSH and LH follicle recipients) was observed [34]; statistical differences were observed in that work.

On the other hand, since there are no statistical differences of this variable in our analysis one might acknowledge the species factor, where there are differences regarding hormone sensitivity [31], which differentiates *Bos indicus* and relevant crossbreeds, which are more sensitive than *Bos taurus* [35]. Taking this into account, one might infer that concentrations of

400 IU of eCG will trigger a different effect on both species; however, such sensitivity was not noticeable in this study, where we worked with *Bos taurus* heifers. Therefore we might conclude it is possible to work with different dosages according to the above factors since dosages of 400 IU of eCG, as used in this research, may be less likely to stimulate the growth of a large number of follicles dosages used in other studies of this subject, which have used 1000 IU [36] and 800 IU [37], and have used both heifers and cows, which could dramatically influence results [38].

When eCG treatment is provided on day 8, follicle dominance may already be established [37] and may only generate stimulation of the dominant follicle (in some cases, the largest subordinate follicle) [31]. In this study, evidence of a dominant follicle was found only for the group of heifers on treatment 2 (eCG day 8), maximum one (1) and minimum one (1), so one might infer the stimulus to this single dominant follicle (see Table 2).

*2.1.2. Dominant follicle diameter, day 9*

All used heifers (treatment 1 – eCG day 5; treatment 2 – eCG day 8 had at least one dominant follicle (follicle ≥ 9 mm). Accordingly, differences were established ($P<0.05$) between both treatments, e.g., a higher follicular diameter for eCG day 5 (treatment 1) (see Table 2). Some researchers working with the same type reported higher values. Sousa et al. [24] applied 400 IU of eCG on day 8 to a group of Holstein cows (in relation to starting day of synchronization), and established a dominant follicle size of 14.7 ± 0.6 mm, compared to 13.1 ± 0.6 mm in cows without eCG. Using Holstein heifers and lactating cows, Sartori et al. [35] established an ovulatory follicle size of 14.9 ± 0.2 mm for heifers, and 16.8 ± 0.5 mm for cows. (Animals with this CL were not treated with any hormone; GnRH was given to others.)

Other studies have applied $PGF_2\alpha$ with an 11-day interval. Lynch et al. [39] used crossbred *Bos taurus* cows, synchronized with application of two dosages of PF2$\alpha$ (an 11-day interval), establishing pre-ovulatory follicles of diameter 14.1 ± 1.9 mm. Thus, based on the results of different studies one might suggest elements such as treatments or dosages used, development stages (heifers, cows), number of births, and so on, may somehow explain the different effects established [40].

Nutrition factors may somehow influence follicular dynamics of heifers [41]. One may suppose that the heifers used in this study were grazing without any additional supplement to guarantee total coverage of their physiological needs; therefore, there might be an imbalance that would generate an initial effect at the hypothalamus, and subsequently the anterior pituitary (adenohypophysis), which affects synthesis and secretion of gonadotropic hormones, and follicle growth and development could be affected [40].

Furthermore, it should be noted that follicle measurements were made on the morning of day 9 in this work (before application of oestradiol benzoate); then, each heifer was given 1 ml (5 mg) of oestradiol benzoate in order to stimulate synthesis and secretion of FSH and LH to facilitate both the final follicle development and ovulation [10]. Therefore, we can suppose that the final development stage of the pre-ovulatory follicle is not yet completed where action by gonadotropic hormones increases final diameter, and follicle diameter averages may be lower

compared to the results of most studies cited, where measurements were performed at the time of maximum follicle development (day 9 in the afternoon) [35] [42].

In relation to the effect (P<0.05) with treatment 1 (eCG day 5 – starting day of synchronized cycle follicle wave) given by this study, eCG seems to trigger an outbreak of event cascade at molecular level, resulting in a further development of dominant follicle [31]. Here we must take into account of course that eCG stimulates the mRNA formation encoding FSH and LH recipients, whose stimulation increases as the follicle is at a very early stage of dominance. During this period eCG continues to operate with a three-day lifespan [43], which virtually guarantees its action during the time that the follicle is becoming dominant, and allows an increased density of FSH and LH recipients, which is associated to follicle growth [44]. On the other hand, compared with heifers of treatment group 2 (eCG day 8), whose application was made with follicle dominance practically established, the stimulating effect of eCG would be very short.

Table 3 shows the results of variables corpus luteum (number) and luteal volume ($mm^3$) on day 17, plasma $P_4$ concentration (ng/ml) on days 9 and 17, and percentage pregnancy (%) on day 52, with a comparison between treatments (eCG day 5 versus eCG day 8). Analysis and discussion of variables by luteal volume on day 17, $P_4$ concentration on day 17, and pregnancy rate on day 52 (see Table 3) are provided to analyse thoroughly the variable pregnancy rate for a greater understanding of studies related that deal with these three variables together.

| Treatment | Corpus Luteum (Number) Day 17 | | | Luteal Volume ($mm^3$) day 17 | | | Progesterone Concentration (ng/ml) | | | | | | Pregnancy (%) Day 52 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Day 9 | | | Day 17 | | | |
| | Min. | Max. | Avg[1] | Min. | Max. | Avg[1] | Min. | Max. | Avg[1] | Min. | Max. | Avg[1] | |
| eCG day 5 (n=42) | 1 | 2 | 1.1 ± 0.3 a | 375 | 16443.6 | 5023.6 ± 512.4 a | 0 | 0.84 | 0.28 ± 0.03 b | 1.98 | 10.1 | 5.12 ± 0.31 a | 69 (29/42) a |
| eCG day 8 (n=28) | 1 | 1 | 1 ± 0 a | 1150.35 | 18057.3 | 5554.9 ± 758.6 a | 0 | 0.86 | 0.33 ± 0.05 b | 2 | 9.6 | 5.52 ± 0.30 a | 64 (18/28) a |
| Total Sample (n=70) | 1 | 2 | 1.1 ± 0.2 | 375.4 | 18057.3 | 5236.1 ± 429.7 | 0 | 0.9 | 0.3 ± 0.03 | 2 | 10 | 5.2 ± 0.22 | 67 (47/70) |

**Table 3.** Performance of luteal structures, progesterone concentration and pregnancy rate. [1]Results by luteal volume and progesterone concentration are expressed as average (mean) ± SEM while other values are expressed as mean ±DS.

### *2.1.3. Corpus luteum (Amount)*

All heifers (70/70) of two experimental groups in this research (42 heifers of treatment 1 –eCG on day 5; 28 heifers of treatment 2 – eCG day 8) had at least one functional corpus luteum consolidated on day 17 (dioestrus stage) with application of control protocols of oestrus cycle proposed (see Table 3); therefore we can suppose that all heifers ovulated, taking into account of course that all of them had at least one dominant follicle on day 9 (see Table 2).

According to the average of this variable there was no difference (P>0.05) between treatments used (Table 3); there were parallels regarding average dominant follicles evidenced (Table 2) during the experiment.

A similar study by Nasser et al. [26] accounted for significant differences between both treatments used (eCG on day 5 or day 8), which provided 400 IU of such a hormone to Nellore/Angus-crossbreed recipients, synchronized for fixed-time embryo transfer, where 1.44 ± 0.18 corpora lutea were obtained (eCG day 5) compared to 1.03 ± 0.03 corpora lutea (eCG day 8), using more sensitive animals in relation to hormone dosages used (*Bos indicus* breed). However, established values in that analysis when eCG was applied on day 8 are similar to this report, maybe due to similar synchronization treatments used.

In a study by Baruselli et al. [37], which used a control treatment (no eCG), statistical differences were evidenced in recipients of Zebu-cross embryos, which were given 800 IU of eCG (day 5 in relation to start of synchronized cycle – growth of a new follicle wave). This study reports 2.58 ± 2.93 corpora lutea in animals with eCG, but 0.5 ± 0.5 corpora lutea in animals without eCG. This finding may demonstrate a super-ovulatory action of eCG following a dosage of 800 IU, given on starting-growth day of the follicle wave. Such a super-ovulatory effect has also been seen in other experiments conducted by Fuentes and De la Fuente [36], who worked with Holstein heifers applying 1000 IU of eCG on day 5 (in relation to treatment start). That study reveals that the hormone significantly increased the number of corpora lutea (between two and five structures for each ovary at the transfer time). Following the variable in several studies, these results could also be indicating different actions of eCG according to dosage used, breed type, and development status of the animal (cow or heifer), which could influence the results for number of follicles (see explanation above) [35].

*2.1.4. Luteal volume*

For this variable there was no difference (P>0.05) between treatment groups (see Table 3). This highlights a synchrony due to evidence of luteal structures established on day 17, so it is evident that 100% of heifers in both treatments (treatment 1 – eCG day 5, 42/42; eCG day 8, 28/28) had at least one consolidated luteal structure. For a better understanding of the results, a discussion related to luteal volume on day 17, $P_4$ concentration on day 17 and pregnancy rate is carried out to analyse the variables by pregnancy rate.

*2.1.5. Progesterone concentrations*

It should be clear that the first day of sampling (day 5) certainly could not have any effect of treatment using eCG for this variable, because at the time of sampling the hormone was not applied to treatment group 1 (eCG day 5); in treatment group 2 eCG was only applied on day 8. However, the behaviour of $P_4$ concentration on day 5 is shown in Figures 4 & 5) in order to analyse trends among groups of heifers. Figure 4 shows ranges between minimum and maximum values, which can be associated to data scattering caused by an irregularity of oestrus-cycle phases, where heifers of the experimental group were subjected to synchronization treatments. This group showed a significant percentage with corpora lutea starting treatments, which might support reports in the literature whereby approximately 40 or 50% of a group of reproductive fit females had luteal structures after carrying out a reproductive examination [44]. Therefore in this analysis we expected a significant percentage of heifers showing $P_4$ levels higher than 1ng/ml (Figure. 4). It should be noted that a slow release device

http://dx.doi.org/10.5772/60425

of $P_4$, applied on day 0 of the control protocol for the oestrus cycle, expedited somehow a concentration (residual) of this hormone [45], evident during the first sampling (day 5). In relation to a $P_4$ concentration on day 9, there was no difference (P> 0.05) among treatments assessed (see Table 3).

It should be noted that on day 5, to start the synchronization protocol, prostaglandin F2$\alpha$ was applied, which triggered a functional and structure regression of any corpus luteum [46]. Therefore, performing an ovarian ultrasound examination during day 9 (sample 2), we did not find any structure consistent with corpora lutea, which was confirmed by observing plasma $P_4$ concentration of heifer groups afterwards. In the absence of luteal structures we could suppose that on day 9 blood $P_4$ (a synchronized cycle) levels should be very close to basal levels, ≤0.3 ng/ml [2].

These basal $P_4$ concentrations (Figure 3) concur with those reported by authors such as Kastelic et al. [47], who worked with a group of Holstein heifers, and related measures of corpus luteum with plasma $P_4$ concentration, resulting on day 9 in a maximum decrease in levels ≤ 0.3 ng/ml. Perry et al. [48], studying a group of crossbred cows with beef characteristics and a hormone therapy applied to synchronize the oestrus cycle (GnRH, $PGF_2\alpha$, GnRH) reported $P_4$ concentration values of 0.2 ng/ml, both in groups of pregnant females and in non-pregnant ones. Similar values were reported by Chagas et al. [49], working with Holstein heifer recipients of embryos, which were not subject to a control treatment of oestrus cycle (embryo transfer with observed heat), 0.21 ± 0.01 ng/ml.

These results by several authors seem consistent to the data of this study, since there are numerical trends which allow us to observe that on the day of maximum follicle development, plasma $P_4$ levels dropped to baseline. This is associated to application of prostaglandin F2$\alpha$ (day 5 of a synchronized cycle), which favours a dramatic reduction of blood flow to the ovary, triggering a cascade of luteolytic mechanisms [50]. Therefore, on day 9 we would expect to find a corpus albicans at ovarian level and therefore $P_4$ baseline [51]. In this study, $PGF_2\alpha$ had a luteolytic effect on both groups of treated females; there were thus no statistical differences in relation to $P_4$ concentration (day 9). As for $P_4$ concentration on day 17, there were no differences (P>0.05) between treatments assessed (see Table 3).

As could be expected at ovary level during third sampling, there is at least one functional and developed corpus luteum whose plasma $P_4$ levels had a tendency to be spread (Figure 4), which is consistent with the difference between sizes of luteal structures shown.

Figure 3 shows an estimated function by regression analysis, which was significant (P<0.05) for a relationship between CL volume and plasma $P_4$ concentration on day 17 (sample 3), regardless of treatments of eCG used (eCG day 5 and eCG day 8) on any heifer used during this work (n=70). Analysis shows a positive correlation between these two variables, indicating that an increased CL volume involves a $P_4$ concentration; therefore a greater $P_4$ involves a greater luteal volume which produced it. Probably, catkins is a possibility to adapt a better embryotrophic environment needed for embryos to be synchronously developed and send signals for maternal recognition, which could possibly trigger higher pregnancy rates [52].

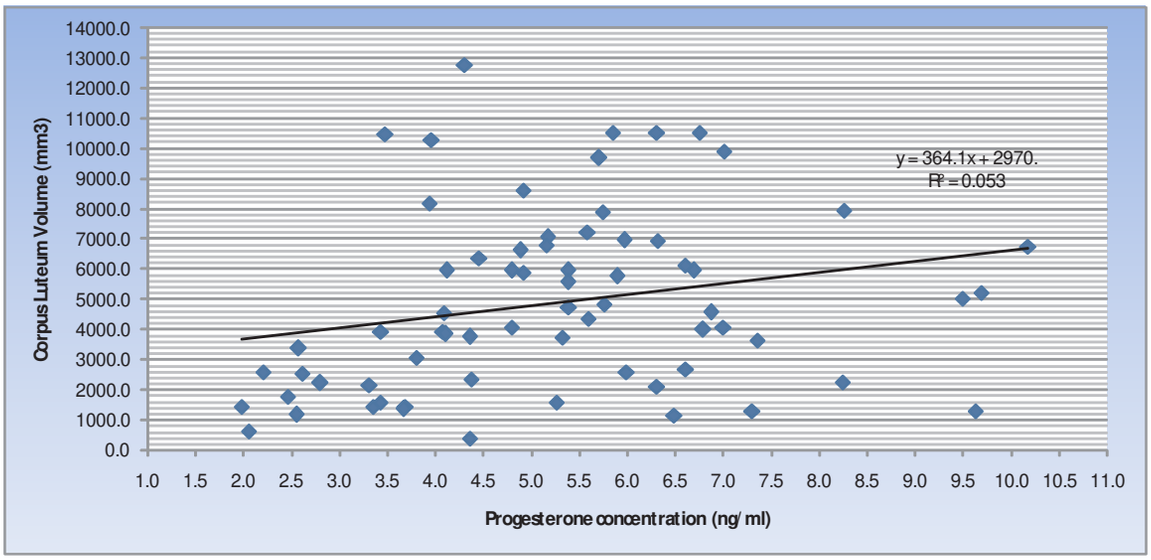


**Figure 3.** Estimated relationship of progesterone concentration (ng/ml) as a function of a luteal volume (day 17).

According to the above, Spain et al. [53] established a correlation ($P<0.001$) between plasma $P_4$ concentration and a luteal area of Holstein cows. The authors proposed that luteal area may be associated to plasma $P_4$ levels and this, in turn, to a pregnancy diagnosis. Sartori et al. [54] working with lactating cows, dry cows and Holstein heifers, gave them $PGF_2\alpha$ on day 7 of the oestrus cycle, also establishing a correlation between luteal volume and plasma $P_4$ concentration. Likewise, Rodriguez et al. [55] in an analysis conducted in the central and low Colombian tropical areas, using several species and crossbreeds, reported a positive correlation between both variables. This also coincides with a report by Duica [44], who worked with embryo recipients, Holstein heifers to which eCG was applied on day 5 (in relation to the synchronized cycle). Other studies have related the corpus luteum weight to the plasma $P_4$ concentration. In this regard, authors such as Mann, [17] using multiparous Holstein cows, established on day 5 (in relation to heat) a strong relationship between the corpus luteum weight and plasma $P_4$ concentration. However, this relationship was not present on day 8 (in relation to heat), unlike in our work.

Other studies have established a variation of this correlation, and authors such as Howell et al. [56] report a seasonal variation. Other authors have also shown a positive correlation between the total CL area and $P_4$ in dairy [57]. However, others have observed insufficient correlation coefficients between these two variables [58] [59].

According to the results presented in several works, authors such as Kastelic et al. [60] suggest that assessment by ultrasonography of corpora lutea becomes a viable alternative to establish $P_4$ concentration for an assessment of a luteal function of Holstein heifers.

In relation to variations reported in some analysis one might say that the amount of luteal tissue formed by small and large luteal cells producing $P_4$ is related to a concentration of the hormone in plasma; however, CLs are not always functional, since the probable cell alterations mentioned or modifications of some inner components may alter their secretion [44]. It should

http://dx.doi.org/10.5772/60425

also be noted that correlation between plasma $P_4$ and luteal area is not constant throughout the oestrus cycle [61]; therefore, during luteal regression phase this index will lose relevance. This has not been reported here because there was indeed a correlation on sampling day 3 (day 17 in relation to the start of treatment synchronization – embryo transfer): a luteal volume of one or more functional CL(s) was detected in all heifers post-ovulating. Other studies have reported similarly on this topic [62] [44].

Figure 4 shows the performance curve by $P_4$ concentration established for three samples conducted with total heifers (n=70) (sample 1 – day 5; sample 2 – day 9; sample 3 – day 17).

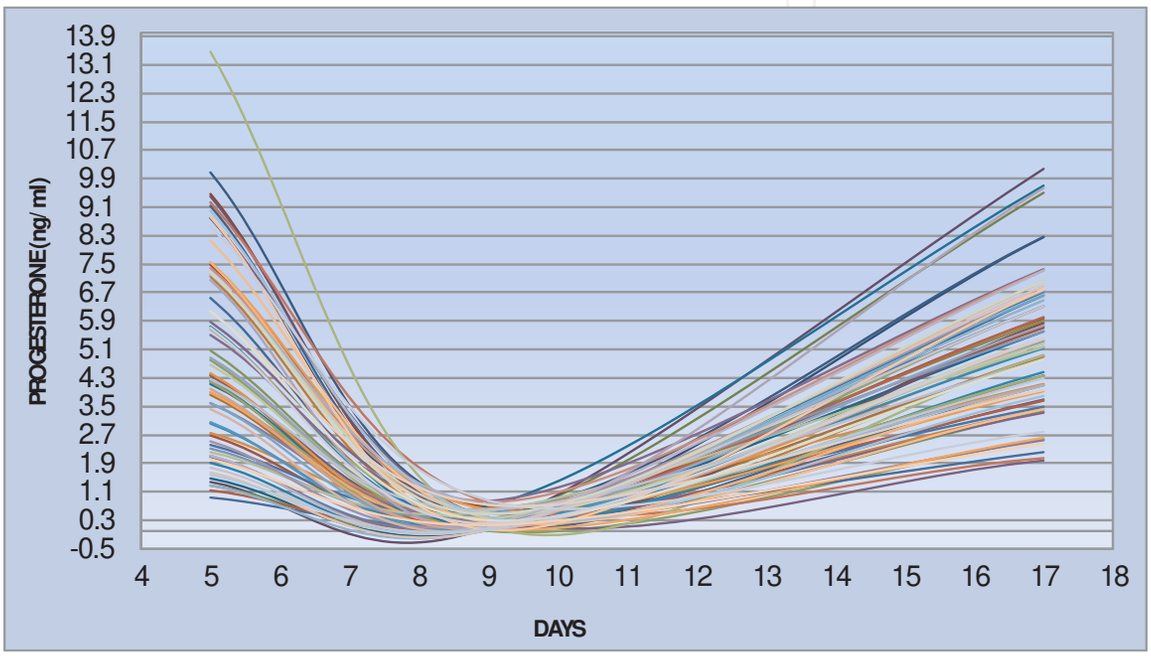


**Figure 4.** Progesterone performance during three samples taken with total heifers (n=70)

In Figure 4, we can infer that the $P_4$ level on day 5 is caused by functional corpora lutea before application of $PGF_2\alpha$, since 98.6% (69/70) of total heifers of this study had ≥1 ng/ml $P_4$ concentration during first sampling (day 5). We might also argue there is a residual effect caused by slow-release implants of $P_4$ on the first sampling day (day 5). Considering that prostaglandin F2$\alpha$ was applied on day 5, immediately after sample 1, the curve (sample 2 – day 9) shows decreasing $P_4$ levels due to a functional and structural luteolysis caused by a luteolytic factor by prostaglandin [46], allowing follicle development and dominance [29]. Figure 4 also shows a behaviour pattern of dispersion in the data range at the beginning and end (days 5 and 17), taking into account the above explanation in relation to possible corpora lutea established at the beginning of the control protocol of oestrus cycle (day zero) and synchronization of wave growth achieved on day 5 [45]. Additionally, controlled ovulation allowed a generation of corpora lutea on day 17 (sample 3), which differed in volume (5236.1 ± 429.7) and was reflected at the end of the curve according to several $P_4$ concentrations established (>1 ng/ml.).

*2.1.6. Pregnancy rate*

In relation to pregnancy percentage there are no differences (P>0.05) between treatments (see Table 3), which is consistent with behaviour established between both variables discussed (luteal volume and plasma $P_4$ concentration). Therefore, the hypothesis *"The day of application of equine Chorionic Gonadotropin (eCG) determines a luteal development, a progesterone concentration, and a pregnancy rate to recipient females of bovine embryos"* is not entirely supported statistically. One might note that the heifers undergoing treatment 1 (eCG day 5) showed 5% more pregnancy than heifers undergoing treatment 2 (eCG day 8), but we cannot legitimately adopt a conclusion.

Conflicting results have been reported in several studies. Authors such as Nasser et al. [26], who used crossbreed embryo recipients (Nellore/Angus – *Bos indicus*/*Bos taurus*), synchronized with a protocol for fixed-time embryo transfer (FTET), changing the eCG application day for each group of animals used (400 IU day 5 or 8), found the following statistical differences of plasma $P_4$ concentration: 2.69 ± 0.38 ng/ml (eCG day 5) versus 1.63 ± 0.21 ng/ml (eCG day 8). Statistical differences in pregnancy percentage (pregnant/transferred) were as follows: 63.4% (eCG day 5) versus 36.1% (eCG day 8), using *in vitro*, freshly transferred embryos. One might point out the lower plasma $P_4$ concentrations established in that study, possibly caused by the small corpora lutea of the typically smaller ovaries of the crossbred Nellore females, i.e., *Bos indicus*, versus the *Bos taurus* used in this study (Holstein heifers).

Machado et al. [62] used Nellore cows (*Bos indicus*) and a protocol for ovulation synchronization with eCG (400 IU) applied on day 9 (synchronized cycle), and obtained 6927.49 ± 05.86 $mm^3$ luteal volume and plasma $P_4$ concentration of 8.15 ± 0.64 ng/ml. These results are higher than those found in this study, but the females in that work were cows, not heifers, which might also influence the results [38].

A plasma $P_4$ concentration higher than that reported here has also been observed in other studies conducted by Marques et al. [63], who set out to analyse the effects of eCG applied at the time of implant removal with $P_4$ in Brangus cows, and reported statistical differences of 8.6 ± 0.4 ng/ml in cows with eCG compared to 6.4 ng/ml in cows without eCG, confirming the previous evidence by Baruselli et al. [37], who synchronized crossbred embryo recipients by applying 800 IU of eCG on day 5, in relation to a synchronized cycle (growth of a new follicle wave), establishing that any eCG application generates larger corpora lutea (>13 mm) on the day of embryo transplant (84% of animals) and a pregnancy rate of 42% (with eCG) versus 34% (without eCG). These results show luteal development may be appropriate when eCG is applied; $P_4$ synthesis could thus be increased, aiding embryonic development. The period of maternal recognition of pregnancy might benefit. This is evident in the pregnancy rates found in previous studies [26] [64].

There may also be discrepancies here with the results of other researchers. For example, Siqueira et al. [65] established no significant differences with crossbred cows (*Bos Taurus*/*Bos indicus*) when applying eCG on day 5 (in relation to start of protocol). In that study, $P_4$ concentration values reported on day 17 (in relation to a synchronized cycle) were 5.2 ± 5.0 ng/ml; the luteal area was 72.4 ± 12.0 $cm^2$ in pregnant cows and 71.4 ± 11.3 $cm^2$ in cows that were

http://dx.doi.org/10.5772/60425

no pregnant. It can be observed that the obtained $P_4$ concentration values were very similar to those obtained in this work – contrary to what we might expect, since one might assume that the ovarian structures and $P_4$ concentration of crossbred cows (*Bos indicus*/*Bos taurus*) would be smaller than those established in our work, i.e., 5.12 ± 0.31 ng/ml, using pure *Bos taurus* females (Holstein heifers). Heifers' structures may be smaller than those in cow females [62].

Working with *Bos indicus* cows, but with no embryo transfer programme or hormone treatments, Aguirre et al. [66] reported 45% pregnancy, a rate similar to other studies, such as that by Fuentes and De la Fuente [36], who used Holstein heifers treated hormonally (1000 IU eCG on day 5 in relation to the treatment start), and reported pregnancy rates with frozen embryos in glycerol of 54%, ethylene glycol 48%, and fresh 52% (no statistical differences). Rodrigues et al. [67], working with recipient cows of Nellore embryos, reported pregnancy rates of 56% (including eCG) compared to 37.8% (not including eCG); these rates are higher than those reported by Pita et al. [68] working with Zebu crossbred heifers, who found rates of 44% with frozen embryos using eCG on day 5 of the protocol, and 30% using eCG on day 8 of the protocol. These figures are lower than those reported by Peixoto et al. [69] using recipient crossbred Zebu and Holstein heifers (*Bos indicus*/*Bos taurus*), where eCG was not applied in synchronization treatment – 63.7%, a similar percentage to that obtained here. Other approaches have verified a range of figures for corpora lutea related to pregnancy rate. Siqueira et al. [65] using a synchronization treatment for fixed-time embryo transfer (FTET) on crossbred recipients (*Bos taurus taurus*/*Bos taurus indicus*) with fresh embryos (eCG applied on day 5 in relation to the protocol start), established a pregnancy rate of 42.9% (in recipients with a single corpus luteum) versus 61.9% (recipients with multiple corpora lutea). They concluded that higher luteal tissue was accompanied by a higher $P_4$ synthesis, and accordingly a higher pregnancy rate, but this effect was not demonstrated in our study.

There are several related studies. Their results are mostly contradictory in relation to the argument that the higher the luteal volume or area, the higher the synthesized plasma $P_4$ concentration, and the higher pregnancy rates [70] [71]. Studies have used different protocols, and some have applied equine Chorionic Gonadotropin (eCG) as hormonal support. In this research, when eCG was provided on day 5 we were trying a follicle recruitment and selection [72] which allows the stimulation of more than one follicle, culminating in multiple ovulation and, in turn, generating greater luteal tissue [73]. This would synthesize a higher $P_4$ concentration [10] and lead to a better embryotrophic environment [19] where embryonic development would be appropriate, favouring the maternal recognition of pregnancy. On the other hand, treatment with eCG applied on day 8, when the follicle domain is established, seeking a greater stimulation of dominant follicles (in some cases the largest subordinate follicle) generates a greater luteal tissue, a greater plasma $P_4$ concentration, and a higher pregnancy rate [73]. Accordingly, one can say that treatment 1 (eCG day 5) and treatment 2 (eCG day 8) are beneficial for pregnancy rates, taking into account the results cited. This result could stem from increased plasma $P_4$ concentration caused by eCG [51], which may have stimulated embryo growth and optimized synchronization and secretion of IFN-τ; therefore, maternal recognition of pregnancy happened at appropriate times [74]. This may have been produced by a luteotrophic action caused by applying 400 IU of eCG [10].

Since there are no differences (P>0.05) between treatments (eCG day 5 vs. eCG day 8) in this research in terms of variables analysed in Table 3 (luteal volume day 17, $P_4$ concentration day 17, pregnancy rate day 52), one might speculate on probable factors which could play an important role during performance of treatments applied in the studies cited. These might include dietary factors among others, which undoubtedly affect physiological reproductive performance [40]. We should also take into account the species factor, by which there are differences in hormone sensitivity [75]. Thus, one might infer that concentrations of 400 IU of eCG will trigger different effects in *indicus*, *taurus* and their crossbreeds [38].

Figure 5 shows an overall average $P_4$ performance for all heifers used in the study with three samples (days 5, 9 and 17). This behaviour is evidenced by the reproductive status of heifers (pregnant or not pregnant), where we can observe significantly higher concentrations during the third sampling (day 17), compared to pregnant heifers – an average of 40.6% (1.585 ng/ml) more plasma $P_4$.

In relation to these concentrations, Siqueira et al. [65] used a synchronization treatment for fixed-time embryo transfer (FTET), which used eCG applied on day 5 (in relation to protocol start) in crossbred recipients (*Bos taurus taurus*/*Bos taurus indicus*). On day of transfer (day 17 related to a synchronized cycle) a $P_4$ concentration of 5.2 ± 5.0 ng/ml in pregnant heifers was established, compared to 3.8 ± 2.4 ng/ml for non-pregnant heifers; these results are similar to those found in our research.

Other works, in which eCG has not been used, as in the study of Chagas et al. [49] who worked with recipient Holstein cows and heifers which were transferred embryos at standing heat, established a $P_4$ concentration on day 0 (day of maximum follicle development) for pregnant females of 0.22 ± 0.01 ng/ml, and for non-pregnant females of 0.21 ± 0.02 ng/ml, values similar to our research. On transfer day (day 17 of this study) these authors established o2.92 ± 0.08 ng/ml for recipient pregnant females versus 2.88 ± 0.08 ng/ml for non-pregnant females. These values are lower than those found in our study, where eCG seems to favour a luteinizing process, influencing $P_4$ synthesis and release [76].

Another study, by Lopes et al. [14], who used Holstein cows synchronized to Ovsynch, established a $P_4$ concentration on day of artificial insemination of 0.19 ± 0.01 ng/ml in pregnant cows and 0.24 ± 0.02 ng/ml in non-pregnant ones, suggesting this hormone could be affecting fertility even at these low concentrations. Moreira et al. [77] suggests low pregnancy rates for cows with an inadequate corpus luteum regression after an injection of $PGF_2\alpha$. This is because $P_4$ concentrations may cause incomplete maturation of pre-ovulatory follicles due to low LH circulating levels (alteration in mode of release), which compromises the follicle ovulation with sensitization of follicle cells changing to luteal status, altering the oocyte release. There could also be a change in $P_4$ secretion, changing the uterine environment so that it may no longer be appropriate for embryonic development; thus the concentration of IFN-τ may not be suitable at the time of the "critical period" to generate an effective signal of maternal recognition of pregnancy due to the asynchrony given in its growth.

As discussed above, our analysis shows that prostaglandin applied on day 5 (in relation to the synchronized cycle) seems to have been effective in all heifers (eCG treatment 1, day 5, and

http://dx.doi.org/10.5772/60425

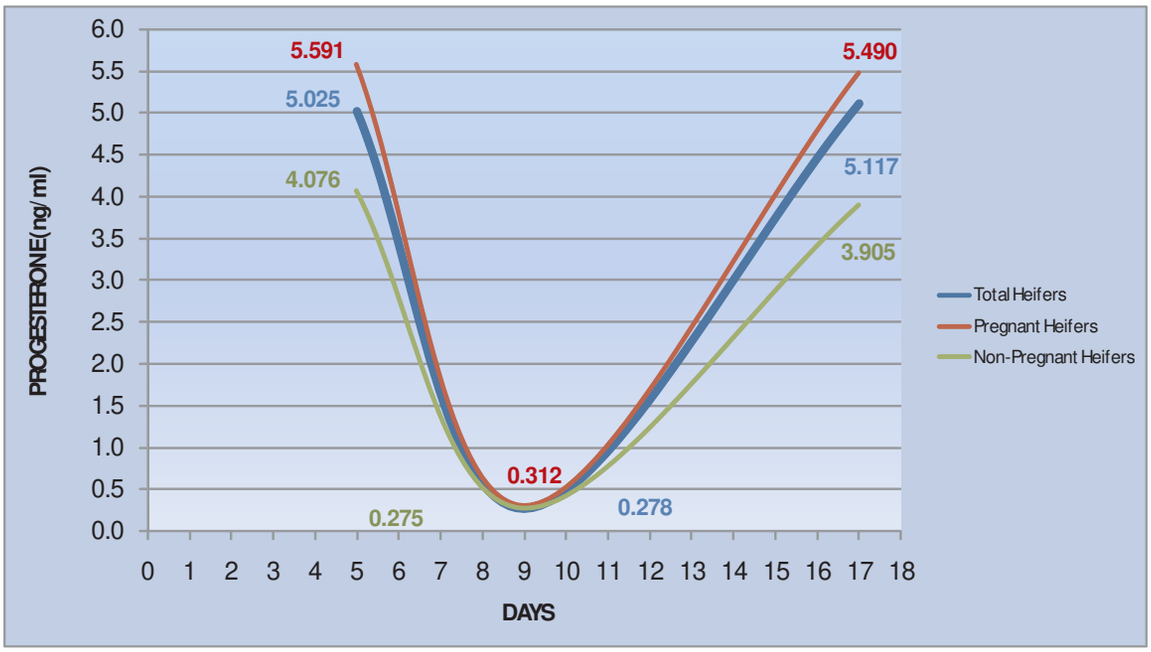


**Figure 5.** Progesterone levels in relation to reproductive status.

eCG treatment 2, day 8), since there was no evidence of luteal structures on the day of sample 2 (day 9 – maximum follicle development). Therefore, one might assume there was a pre-ovulatory LH peak, taking into account of course the measurable concentration of $P_4$ on that day (day 9); one might also assume that this came from a luteinizing process during a dominance period of the ovulatory follicle [2] or a residual status of the $P_4$ implant used [32], for example. In conclusion, $P_4$ may directly affect LH synthesis and release [78], which is evident from Figure 6.

Moreover, in some cases plasma $P_4$ concentration on day of embryo transfer (day 17) was higher (numerically) in successfully pregnant females compared to those not pregnant (Figure 5). In this context, Duica [44] has shown a potential impact upon recipient Holstein heifers treated with eCG on day 5 (in relation to treatment synchronization start), establishing differences ($P<0.05$) between pregnant and non-pregnant females, which might somewhere involve appropriate support in the uterine environment provided by $P_4$ to the embryos transferred, which are efficient in IFN-τ synthesis and secretion throughout their development [19], aiding maternal recognition of pregnancy [18].

Figure 6 shows $P_4$ concentration on day 9 of 33% (23/70) in successfully pregnant heifers. It is evident that 17% (4/23) of these heifers showed $P_4$ concentration lower than 0.1 ng/ml. In contrast, 83% (19/23) of heifers had a $P_4$ concentration higher than 0.1 ng/ml.

Of the 23 heifers that did not successfully become pregnant, 57% (13/23) belonged to treatment group 1 (eCG day 5) and 43% (10/23) to treatment group 2 (eCG day 8). Of those in group 1 (eCG day 5) on day 9, 15% (2/13) showed decreased $P_4$ concentration at values lower than 0.1 ng/ml, while 85% reported higher concentrations. Of those in group 2 (eCG day 8) on day 9,

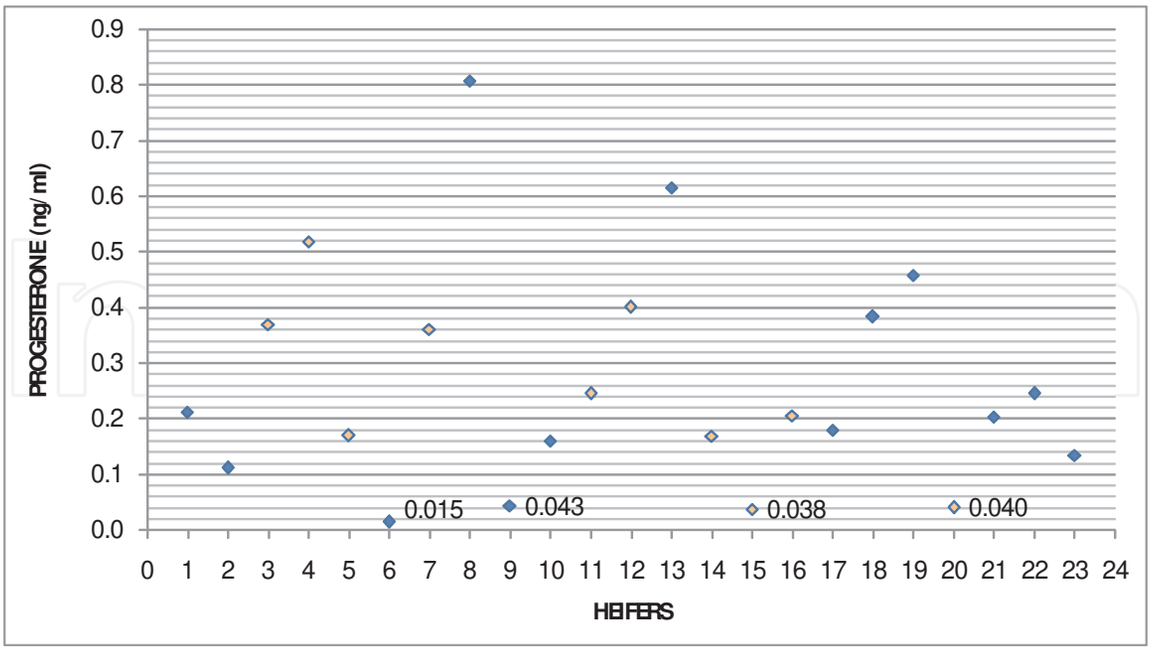


**Figure 6.** Progesterone concentration – non-pregnant heifers (sample 2 - day 9).

20% (2/10) showed decreased $P_4$ concentration at values lower than 0.1 ng/ml, while 80% reported higher concentrations.

Based on these results, we can suppose that a stronger decrease of $P_4$ on the day of maximum follicle development (sample 2 – day 9) to minimum levels should improve pregnancy rates. This is because the expected LH peak may occur without difficulty with subsequent, appropriate luteinization and ovulation [79]; cows that present supra-basal $P_4$ levels may not become fertilized. Additionally, this may affect ovulatory follicle luteinization, and thus the life structuring or programming of the corpus luteum and $P_4$ synthesis. Finally, it should be noted that the uterus might also be damaged – there is evidence of imbalance between the steroid hormones $P_4$ and oestradiol, for example [80].

Figure 7 shows logistic function according to the treatment effect (eCG day 5 and eCG day 8) of follicle diameter (dominant follicle – day 9), total luteal volume (day 17), and progesterone concentration levels (day 17) upon pregnancy diagnosis. Only plasma progesterone concentration on day 17 (third sample) was significant ($P<0.1$) in terms of pregnancy probability.

As we can observe (10.1 ng/ml) one might infer there is an increasing probability of pregnancy to the maximum $P_4$ concentration value. We should also note that this increased probability of pregnancy may increase to the maximum $P_4$ level for the total animals used in this research (treatment 1, eCG day 5; treatment 2, eCG day 8) in the range of values reported; however, we cannot always expect that an increased $P_4$ value increases the likelihood of pregnancy [25]. This can happen regardless of indications that blood $P_4$ levels and uterine environment during luteal phase favour pregnancy establishment and maintenance conditions [51].

In contrast to the results of this research, other works have not shown any significant effect of $P_4$ upon pregnancy. Siqueira et al. [65] used a synchronization treatment for a fixed-time

http://dx.doi.org/10.5772/60425

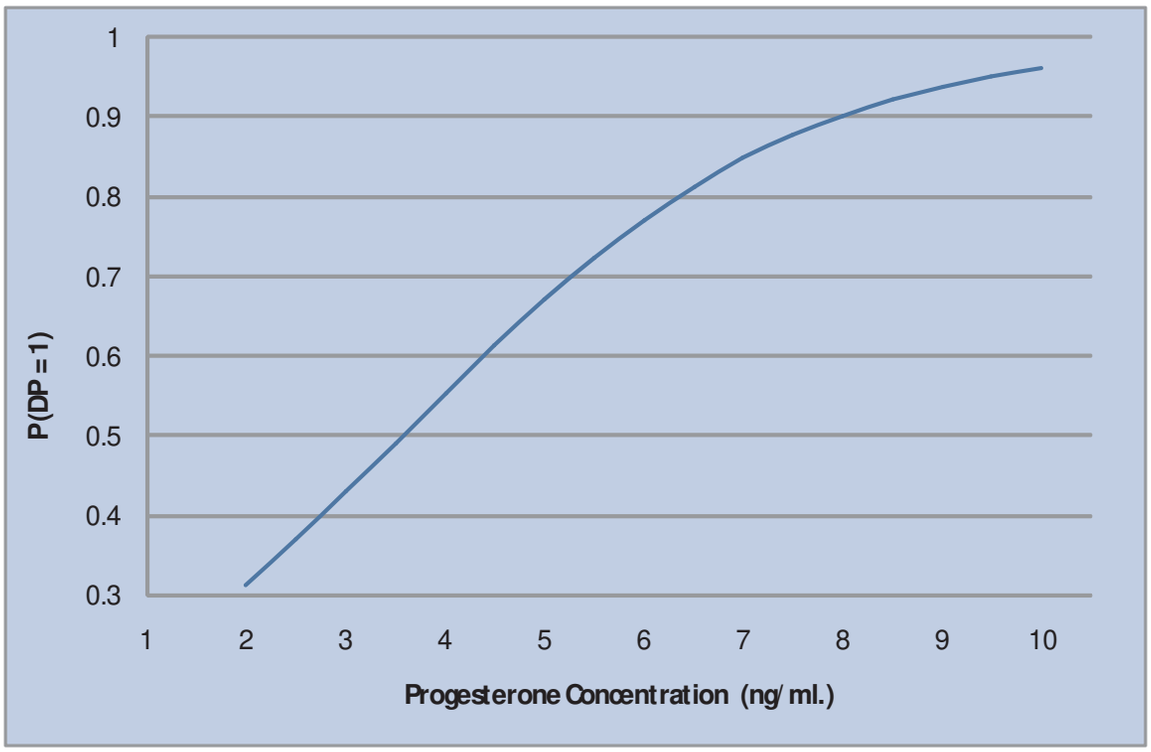


**Figure 7.** Logistic function for the range of values of progesterone concentration

embryo transfer (*in vivo* and *in vitro* embryos), applying eCG on day 5 (in relation to the protocol start) in crossbred recipients (*Bos taurus taurus*/*Bos taurus indicus*), applying a logistic regression model, and showed that the only variable (P=0.0002) significantly affecting pregnancy was the embryo type produced (*in vivo* and *in vitro*), not $P_4$ concentration, corpus luteum echo texture and area, animal category, embryo quality, or embryo development stage.

Rodriguez et al. [55] used several species and crossbreeds as recipients with transferred fresh and frozen embryos, then assessed the effect of other independent variables (CL diameter, $P_4$ concentration, embryo stage, embryo quality and species) upon pregnancy diagnosis. They did not establish any effect of any of these variables upon pregnancy diagnosis.

Other works have tried to relate pregnancy to other variables. For example, Peixoto et al. [69] used crossbred Holstein and Zebu heifers (*Bos indicus*/*Bos taurus*) as embryo recipients to determine which explanatory variables (transfer years: 1992-1999; season: autumn, winter and spring; embryo species; embryo stage; embryo quality; and synchrony between donors and recipients) might have a direct impact upon pregnancy. They established that the best logistic model to explain pregnancy included effects of year, transfer time, embryo stage, quality, and oestrus synchrony between donor and recipient.

Including other explanatory variables, Perry et al. [42] worked with synchronized crossbred heifers under a CO-Synch protocol for artificial insemination, and established that a pre-ovulatory follicle size (≥12.8 mm) predicted pregnancy at about 68.0 ± 4.9%, which decreased with follicle size.

Table 4 shows $P_4$ profile comparisons (ng/ml) on the third sampling (day 17) using the Odds ratio, according to the results obtained by performing the logistic regression proposed.

| Plasma Progesterone Concentration Profiles (ng/ml) | | Pregnancy Probability |
|---|---|---|
| Profile 1 | Profile 2 | |
| 6 | 4 | 2.72 |
| 5 | 2 | 4.48 |
| 4 | 2 | 2.72 |
| 8 | 4 | 7.4 |

**Table 4.** Contrasting progesterone profiles – pregnancy Odds ratio

Based on the results shown in the table, we might compare a heifer with a $P_4$ concentration of 6 ng/ml (profile 1) to a heifer with a $P_4$ concentration of 4 ng/ml (profile 2), and establish an Odds ratio of 2.72, indicating that it is 2.72 times more likely that the profile-1 cow should become pregnant than the profile-2 cow; therefore, pregnancy probability increases in line with $P_4$ concentration.

## 3. Conclusions

This study allows us to conclude that:

There was no effect of the day of application of eCG (day 5 or 8) on the number of dominant follicles; however, the eCG did affect the follicular diameter when applied on day 5, relating to the start of an oestrus synchronization protocol.

There was no effect of the day of application of eCG on the plasmatic progesterone levels on days 9 and 17 in protocols of oestrus synchronization in Holstein heifers. In the same way, the day of application of eCG does not affect the pregnancy rate evaluated on day 52 in Holstein heifers.

Further works should focus on antiluteolitic strategies that allow pregnancy rates in heifer and cow recipients involved in embryo transfer programmes to be improved.

http://dx.doi.org/10.5772/60425

## Author details

Néstor Isaías Tovío Luna*, Arturo Duica Amaya and Henry Alberto Grajales Lombana

*Address all correspondence to: nitoviol@unal.edu.co

Groups Researchers Biologia de la adaptacion de los animales al tropico, Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional. UYWAY, Facultad de Ciencias Agrarias, programa de Zootecnia, Fundacion Universitaria Agraria de Colombia, Bogota, Columbia

## References

[1] Santos J, Thatcher W, Chebel R et al. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Animal Reproduction Science. 2004; 83: pp. 513-535.

[2] Grajales H, Tovío N, Duica A. *Fundamentos de fisiología reproductiva en la hembra bovina*. 1 ed. Bogota, D.C: Editorial Universidad Nacional de Colombia; 2011

[3] Binelli M, Thatcher W, Mattos R, Baruselli P. Antiluteolytic Strategies to Improve Fertility in Cattle. Theriogenology. 2001; 56: pp. 1451-1463.

[4] Silke V, Diskin M, Kenny D et al. Extent, pattern and factors associated with late embryonic loss in dairy cows. Animal Reproduction Science. 2002; 71: pp. 1-12.

[5] Grajales H, Tovío N. Aspectos Básicos del ciclo estral de la vaca. Revista Genética Bovina. 2009; 15: pp. 32-36.

[6] Tovío N, Duica A, Grajales H. Mortalidad embrionaria en bovinos. Revista Genética bovina. 2010; 19: pp. 34-38.

[7] Tovío N, Duica A, Grajales H. Desarrollo embrionario y estrategias antiluteoliticas hormonales en programas de transplante de embriones bovinos. Revista MVZ Córdoba. 2008; 13 (1): pp. 1240-1251.

[8] Silva R, Rodriguez C, Marques M et al. Efeito do eCG e do GnRH na taxa de prenhez de vacas Nelore lactantes inseminadas em tempo fixo. Acta Scientiae Veterinariae. 2004; 32 (Suplemento): pp. 221.

[9] Duica A, Tovío N, Grajales H. Factores que afectan la eficiencia reproductiva de la hembra receptora en un programa de transplante de embriones bovinos. Journal de Medicina Veterinaria Universidad de la Salle. 2007 Jul; 14: pp. 107-124

[10] Baruselli P, Bó G, Reis E et al. Introducción de la IATF en el manejo reproductivo de rebaños de ganado de engorde en Brasil. Congreso Internacional de Reproducción Bovina Intervet. 2005; Bogotá, Colombia.

[11] Figueira M, Pegorer R, Ereno R et al. Neither plasma progesterone concentrations nor exogenous eCG affects rates of ovulation or pregnancy in fixed-time artificial insemination (FTAI) protocols for puberal Nellore heifers. Theriogenology. 2011; 75: (1): pp. 17-23.

[12] Peña M, Góngora A, Estrada J. Factores de crecimiento en el desarrollo folicular, embrionario temprano e implantación. Implicaciones en la producción de embriones bovinos. Journal MVZ Córdoba. 2007; 12 (1): pp. 942-954.

[13] Tovío N. Efectos de la aplicación de eCG (Día 5 u 8) sobre del cuerpo lúteo, nivel de progesterona y tasa de preñez en hembras receptoras de embriones bovinos. Tesis de Maestria. Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia; 2011.

[14] Lopes A, Butler S, Gilbert R et al. Relationship of pre-ovulatory follicle size, estradiol concentrations and season to pregnancy outcome in dairy cows. Animal Reproduction Science. 2007; 99: pp. 34-43.

[15] Moreira F, Badinga L, Burnley C et al. Bovine somatotropin increases embryonic development in super ovulated cows and improves post-transfer pregnancy rates when given to lactating recipient cows. Theriogenology. 2004; 57: pp. 1371-87.

[16] Kerbler T, Buhr M, Jordan L et al. Relationship between maternal plasma progesterone concentration and interferon-tau synthesis by the conceptus in cattle. Theriogenology.1997; 47: pp. 703-714.

[17] Mann G. Corpus luteum size and plasma progesterone concentration in cows. Animal Reproduction Science. 2009; 115: pp. 296-299.

[18] Vasconcelos J, Sartori R, Oliveira H et al. Reduction is size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology. 2001; 56: pp. 307-314.

[19] Gonella A, Grajales H, Hernández A. Ambiente receptivo uterino: control materno, control embrionario, muerte embrionaria. Journal MVZ Córdoba. 2010; 15 (1): pp. 1976-1984.

[20] Filho M, Ayres H, Ferreira R et al. Equine chorionic gonadotropin and gonadotropin-releasing hormone enhance fertility in a norgestomet-based, timed artificial insemination protocol in suckled Nellore (*Bos indicus*) cows. Theriogenology. 2010; 73: pp. 651-658.

[21] Bousfield G, Butnev V. Identification of twelve O-glycosylation sites in equine chorionic gonadtropin beta and equine luteinizing hormone by solid-phase Edman degradation. Biology Reproduction. 2001; 64: pp. 136-147.

[22] Forcada F, Ait Amer-Meziane J, Abecia M et al. Repeated super ovulation using a simplified FSH/eCG treatment for in vivo embryo production in sheep. Theriogenology. 2011; 75 (4): pp. 769-776.

[23] Everton L and Baruselli P. Sincronización de receptoras cruce por cebú en condiciones tropicales. IV Seminario de Reproducción de Grandes Animales, CGR, Biotecnología Reproductiva, Bogotá, Colombia, 2003.

[24] Souza A, Viechnieski S, Lima F et al. Effects of equine chorionic gonadotropin and type of ovulatory stimulus in a timed-AI protocol on reproductive responses in dairy cows. Theriogenology. 2009; 72: pp. 10-21.

[25] Melanie J, Starbuck A, Dailey E et al. Factors affecting retention of early pregnancy in dairy cattle. Animal Reproduction Science. 2004; 84: pp. 27-39.

[26] Nasser L, Reis E, Oliveira M et al. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. Animal Reproduction Science. 2004; 82: pp. 479-486.

[27] Quesada J and Ortiz J. Sincronización de receptoras de embriones tratadas con CIDR, benzoato de estradiol, D Cloprostenol asociado o no a eCG. VII Simposio internacional de reproducción animal – IRAC; Córdoba, Argentina. 2007.

[28] Campanile G, Di Palo R, Neglia G. et al. Corpus luteum and embryonic mortality in buffaloes treated with a GnRH agonist, hCG and progesterone. Theriogenology. 2007; 67: pp. 1393-1398.

[29] Baruselli P, Marques O, Nasser L et al. Effect of eCG on pregnancy rates of lactating zebu beef cows treated with CIDR–B devices for timed artificial insemination. Theriogenology. 2003; 59: p. 214.

[30] Berisha, B. and Schams, D. Ovarian function in ruminants. Domestic Animal Endocrinology. 2005; 29: pp. 305-317.

[31] Knobil E and Neill J. *Physiology of reproduction*. Third ed. Elsevier. United States of America. 2006.

[32] Bó G, Colazo M, Martínez M et al. Sincronización de la emergencia de la onda folicular y la ovulación en animales tratados con progestágenos y diferentes esteres de estradiol. 2o Simposio Internacional de Reprodução Animal Aplicada; Córdoba, Argentina. 2006.

[33] Kuran M, Hutchinson J, Broadbent P. The response of bovine granulosa cells to different gonadotrophins in culture. Animal Reproduction Science. 1991; 45: pp. 1-12.

[34] Cameron A, Batt P. PMSG may directly stimulate ovulation in female goats. Animal Reproduction Science. 1991; 25: pp. 233-239.

[35] Sartori R, Haughian J, Shaver R et al. Comparison of Ovarian Function and Circulating Steroids in Estrous Cycles of Holstein Heifers and Lactating Cows. Dairy Science. 2004; 8: pp. 905-920.

[36] Fuentes S, De la Fuente J. Tasas de gestación de novillas receptoras sincronizadas con Gonadotropina Coriónica Equina u Hormona Folículo Estimulante. Acta Scientiae Veterinariae. 2007; 35 (3): pp. 767-772.

[37] Baruselli P, Marques M, Hothnann E et al. Increased pregnancy rates in embryo recipients treated with CIDR-B devices. Theriogenology. 2011; 55, p. 157.

[38] Nogueira M, Melo D, Carvalho L et al. Do progesterone concentrations decrease pregnancy rates in embryo recipients synchronized with PGF2 and eCG? Theriogenology. 2003; 61 (7): pp. 1283-1290.

[39] Lynch C, Kenny D, Childs S et al. The relationship between periovulatory endocrine and follicular activity on corpus luteum size, function, and subsequent embryo survival. Theriogenology. 2010; 73: pp. 190-198.

[40] Rekwot P. Effects of feeding maize stover and cottonseed cake on onset of puberty in Bunaji (*Bos indicus*) heifers. Tropical Animal Health and Production. 2004; 36: pp. 637-644.

[41] Roche J. The effect of nutritional management of the dairy cow on reproductive efficiency. Animal Reproduction Science. 2004; 96: pp. 282-296.

[42] Perry G, Smith M, Roberts A et al. Relationship between size of the ovulatory follicle and pregnancy success in beef. Journal Animal Science. 2005; 85: pp. 684-689.

[43] Bó G, Alonso A, Caicedo J et al. Actualización sobre fisiología de la reproducción de la vaca. Especialización en reproducción bovina. IRAC, CGR, Universidad de Córdoba, Argentina; 2004.

[44] Duica a. Efecto del diámetro del folículo ovulatorio, tamaño del cuerpo lúteo y perfiles de progesterona sobre la tasa de preñez en la hembra receptora de embriones bovinos. Tesis de Maestria. Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia; 2011.

[45] Bó G. Sincronización de celos para programas de Inseminación Artificial y Transferencia de embriones Bovinos. Instituto de Reproducción Animal de Córdoba Argentina; 2003.

[46] Barnea E, Choi Y and Leavis P. Embryo maternal signaling prior to implantation. In: Textbook of Obstetrics Gynecology, 1 ed. Boston; pp.64. 2005.

[47] Kastelic J, Pierson R, Ginther O. Ultrasonic Morphology of a corpora lutea and central luteal cavities during the estrous cycle and early pregnancy in heifers. Theriogenology.1990; 34 (3): pp. 487-498.

[48] Perry G, Smith M, Lucy M et al. Proceedings of the national academy of sciences of the United States of America Current (PNAS). 2005; 102 (14), pp. 5268-5273.

[49] Chagas J, Lopes L, Robalo J. Plasma progesterone profiles and factors affecting embryo-fetal mortality following embryo transfer in dairy cattle. Theriogenology. 2002; 58: pp. 51-59.

[50] Sakase M, Kawate N, Nakagawa C, Fukushima M, Noda M, Takeda K, Ueno S, Inaba T, Kida, K and Tamada H. Preventive effects of CIDR-based protocols on premature ovulation before timed-AI in Ovsynch in cycling beef cows. The Veterinary Journal. In press; 2006.

[51] Shams D, Berisha B. Regulations of corpus luteum function in cattle – an overview. Reproduction Domestic Animals. 2004; 39: pp. 241-251.

[52] Gonella A. Posible relación de los niveles séricos ováricos (17 Beta estradiol y Progesterona), sus respectivos receptores endometriales (ER-1 alfa y PGR) y los cambios histológicos del endometrio durante el ciclo estral en hembras cíclicas de tres razas bovinas. Tesis de Maestria. Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia; 2011.

[53] Silva R, Rodriguez C, Marques M et al. Efeito do eCG e do GnRH na taxa de prenhez de vacas Nelore lactantes inseminadas em tempo fixo. Acta Scientiae Veterinariae. 2004; 32: pp. 221.

[54] Sartori G, Rosa M, Wiltbank M. Ovarian Structures and Circulating Steroids in Heifers and Lactating Cows in Summer and Lactating and Dry Cows in Winter. Journal Dairy Science. 2002; 85: pp. 2813-2822.

[55] Rodríguez J, Giraldo J, Castañeda S et al. Análisis multifactorial de las tasas de preñez en programas de transferencia de embriones en Colombia. Revista MVZ Córdoba. 2007; 12 (2): p. 978.

[56] Howell J, Fuquay J, Smith A. Corpus luteum growth and function in lactating Holstein cows during spring and summer. Journal Dairy Science. 1994; 77, pp. 735-739.

[57] Sprecher D, Nebel R, Whitmn S. The predictive value, sensitivity and specificity of palpation per rectum and transrectal ultrasonography for the determination of bovine luteal status. Theriogenology. 1998; 31: pp. 1165-1172.

[58] Badinga L, Thatcher W, Wilcox C et al. Effect of season on follicular dynamics and plasma concentrations of estradiol-17 beta, progesterone and luteinizing hormone in lactating Holstein cows. Theriogenology.1994; 42: pp. 1263-1274.

[59] Viana J, Torres C, Fernandes C et al. Avaliaçao ultra-sonográfica do corpo lúteo em novilhas mestiças utilizadas como receptoras de embriao. Archivos de Reproducción Animal. 1998; 5: pp. 42-47.

[60] Kastelic P, Bergfelt D, Ginther O. Relationship between ultrasonic assessment of the corpus luteum and plasma progesterone concentration in heifers. Theriogenology. 1990; 33 (6): pp. 1269-1278.

[61] Tom J, Pierson R, Adams G. Quantitative echotexture analysis of bovine ovarian follicles. Theriogenology. 1998; 50: pp. 339-346.

[62] Battocchio M, Gabai G, Mollo A et al. Agreement between ultrasonographic classification of the CL and the plasma progesterone concentration in dairy cows. Theriogenology. 1999; 51: pp. 1059-1069.

[63] Marques M, Reis E, Filho E et al. Efeitos da adminiatracao de eCG e de benzoato de estradiol para sincronizacao da ovulacao em vacas Bos taurus taurus X Bos taurus indicus no periodo pós parto. Simposio Internacional de Reproducción Animal; Huerta Grande; 2003.

[64] Bó G. and Cutaia L. Implementación de programas de inseminación artificial en rodeos de cría. Argentina. VI Simposio Internacional de Reproducción Animal. Instituto de Reproducción Animal; Córdoba Argentina. 2005; pp. 326-332.

[65] Siqueira L., Torres C, Souza E et al. Pregnancy rates and corpus luteum-related factors affecting pregnancy establishment in bovine recipients synchronized for fixed-time embryo transfer. Theriogenology. 2009; 72: pp. 949-958.

[66] Aguirre G, Pardo C, Góngora A. Inicio del celo, tasa de gestación y relación del tiempo de inseminación con los niveles de progesterona en vacas brahmán. Revista MVZ Córdoba. 2006; 11 (1): pp. 766-772.

[67] Rodrigues C, Ayres H, Reis E et al. Aumento de taxa de prenhez em vacas Nelore inseminadas em tempo fixo com o uso de eCG em diferentes periodos pós-parto; Acta Scientae Veterinariae. 2004; 32: p. 220.

[68] Pita F, Matute R, Tribulo R et al. Efecto de la aplicación de la Gonadotropina Coriónica Equina (eCG) y PGF2 alfa sobre la tasa de aprovechamiento y tasa de preñez en un protocolo de transferencia de embriones a tiempo fijo. VIII Simposio internacional de reproducción animal – IRAC; Córdoba, Argentina; 2009.

[69] Peixoto M, Bergmann J, Suyama E et al. Logistic regression analysis of pregnancy rate following transfer of *Bos indicus* embryos into *Bos indicus*/*Bos taurus* heifers. Theriogenology. 2007; 67: pp. 287-292.

[70] Moura M, Marques M, Frare J et al. Sincronizacao da ovulacao com Crestar ® e CIDR ® para inovulacao de embrioes bovinos em tempo fixo. VII Simposio internacional de reproducción animal; Cordoba, Argentina; 2001.

[71] Marques M et al. Follicular dynamics and pregnancy rates in Bos Taurus xBos indicus embryo transfer recipients treated to increase plasma progesterone concentrations; 2012. 9: p. 11-119.

[72] Barret D, Bartlewski P, Duggaathi R et al. Suppression of follicle wave emergence in cycle ewes by supraphysiologic concentrations of estradiol 17 beta and induction with a physiologic dose of exogenous ovine follicle stimulating hormone. Biology Reproduction. 2006; 75: pp. 633-641.

[73] Bó G. and Caccia M. *Ultrasonografía reproductiva en el ganado bovino*. Argentina. Instituto de Reproducción Animal de Córdoba (IRAC); 2003.

[74] Mann G, Lamming G. The influence of progesterone during early pregnancy in cattle. Reproduction Domestic Animals. 1999; 34: pp. 269-274.

[75] Sartori R, Barros A. Reproductive cycles in *Bos indicus* cattle. Animal Reproduction Science. 2011; 124 (3): pp. 244-250.

[76] Niswender G, Juengel J, Silva P et al. Mechanisms controlling the function and life span of the corpus luteum. Physiological Reviews. 2000; 222 (80): pp. 1-29.

[77] Moreira F, Risco C, Pires M et al. Use of bovine somatotropin in lactating dairy cows receiving timed artificial insemination. Journal Dairy Science. 2000; 83: pp. 1237-1247.

[78] Olivera M. Gestación. In: Galina C. Reproduccion de los animals domesticos. 1 ed. Mexico: Limusa. 2006; pp. 165-187.

[79] Lucy M, Savio J, Badinga L et al. Factors that affect ovarian follicular dynamics in cattle. Journal Animal Science. 1992; 70: pp. 3615-3626.

[80] Drawash A. The importance of milk progesterone concentrations during early pregnancy in the cow. Journal of Animal Breeding. 1998; 2: pp. 41-43.

IntechOpen