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**Effects of GnRH or hCG on day 11 after artificial insemination in cows luteal activity**

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**ABSTRACT.** In order to optimize luteal function, human chorionic gonadotrophin (hCG) or gonadotrophin releasing hormone (GnRH) were used on day 11 after artificial insemination (AI). 33 cows synchronized by the Ovsynch and divided into 3 groups according to the type of treatment: 1) hCG (1500 IU, n=11); 2) GnRH (100 µg, n=11) and 3) control (2 mL of saline, n=11). Blood samples were collected from all animals every 3 days from day 5 to day 23 to determine progesterone concentration. Ultrasonography was used to monitor the luteal surface structures at the time of blood sample collection. An accessory corpus luteum (CL) formed in 63.63% of cows treated with GnRH or hCG, resulting in an increase in the total luteal tissue area compared with the controls. Compared with the controls, the principal CL area was increased by hCG but not by GnRH. Additionally, compared with the control group, hCG-treated cows had increased progesterone concentrations ( $p < 0.0001$ ), while GnRH-treated cows had P4 similar to that of controls cows.

**Keywords:** hCG, GnRH, Progesterone, Accessory corpus luteum, Cow.

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## INTRODUCTION

Early embryonic loss is the principal cause of low pregnancy rate (Inskeep and Dailey 2005). Indeed, embryonic and foetal mortalities are estimated to be 50% in dairy cows and between 70 and 80% of these losses occur in the first 16 days after AI (Diskin et al., 2011). One of the causes of embryonic loss is related to delayed embryo development, which reduces signalling for the maternal recognition of pregnancy (Mann and Lamming 2001). This condition may be due to a lower progesterone concentration in dairy cows (Mann and Lamming 2001; Wiltbank et al., 2011). Progesterone plays an important role in regulating changes in the uterine environment conducive to the development of the embryo and maintenance of pregnancy (Geisert et al., 1992). Increased milk production in dairy cows is connected with increased general metabolism as well as with increased progesterone metabolism in the liver, which decreases progesterone concentration in peripheral blood (Rhinehart et al., 2009). Progesterone insufficiency can be an important factor responsible for the decrease of the conception rate and is associated with abnormal early embryo development (Mann and Lamming 2001), while high progesterone concentration favours embryo development and interferon- $\tau$  secretion (Lonergan 2011). Several approaches have been used to increase the concentration of progesterone in the blood in order to reduce the occurrence of embryo death (Campanile et al., 2007). In different studies, progesterone has been administered via implants or intra-vaginal devices with inconsistent results (Wiltbank et al., 2014). Other studies have shown that administration of natural sequence GnRH, GnRH agonists or hCG after AI can stimulate CL function which induces accessory CL formation, increases progesterone concentration, and reduces oestradiol production with a consequent positive effect on embryonic survival (Thatcher et al., 2003). Administration of GnRH, a GnRH agonist or hCG after AI at specific times, coincident with the presence of the dominant follicle of the first and second follicular waves, may stimulate CL function, induce accessory CL formation, increase progesterone concentration (Schmitt et al., 1996a, Stevenson et al., 2007) and reduce oestrogen production with a consequent positive effect on pregnancy rate or embryo survival (Thatcher et al., 2003; Šuluburić et al., 2017). Several studies have investigated the effects of GnRH

administered during mid-estrous cycle (day 12, 13 or 14 after AI) (Szenci et al., 2006; Lopez-Gatius et al., 2006). Beneficial effects of treatment with GnRH at day 11-12 after AI on fertility have been observed in lactating dairy cows during heat stress (Lopez-Gatius et al., 2006), but very few studies that have attempted to compare GnRH and hCG injected in the mid-cycle to improve luteal function after AI. We hypothesized that cows receiving GnRH in day 11 of estrous would have concentrations of progesterone closer to cows receiving hCG. The objective of this study was to compare the effects of GnRH and hCG administration at day 11 after AI on CL development and function, in terms of progesterone secretion in cows.

## MATERIALS AND METHODS

### Ethical approval

Experimental procedures were approved by the Institutional Animal Care Committee of the National Administration of the Algerian Higher Education and Scientific Research (Ethical approval number: 98-11, Law of August 22, 1998).

### Animals and treatments

The experiment was undertaken at a commercial dairy farm in Mitija (longitude 36° and latitude 3°), Algeria from January to April. Animals were housed in free-stall barns and fed a diet consisting of grass and clover supplemented with a commercial concentrate (18% digestible raw protein), as well as roughly crushed maize grains, soybean meal, barley and vitamin–mineral mixture. Cows were selected by clinical examination before AI and only those in a healthy reproductive status were included in the study. A total of 33 primiparous Holstein lactating cows (ranging in age from 2-3 years old) were selected. Cows were synchronized by the Ovsynch protocol initiated with Gonadorelin (Cystoreline® CEVA, France) followed by an injection of prostaglandin PGF2 $\alpha$  analogue at day 7 (Enzaprost® CEVA, France), and two days later, the cows received a second Gonadorelin injection. All animals were inseminated 12–16 h after the second injection of Gonadorelin (day 0). Eleven days after AI (day 11), cows were randomly assigned to one of the three treatment groups, administered a single injection of 1500 IU hCG (hCG group, n=11, Chorulon® Intevet Laboratories

Ltd Holland given i.v.), 100 µg GnRH (GnRH group, n=11, Cystoréline® CEVA France given i.m.) or 2 mL of 0.9% saline (n=11, control group).

#### Blood collection procedures and progesterone quantification

Blood samples were collected via venipuncture of the median caudal vein or artery into 5 mL evacuated tubes (VACUETTE® Blood Collection Tubes) every 3 days from day 5 to day 23 after timed AI (Fig 1) for later analysis of serum progesterone; the first sample collected immediately before administration of treatments. Blood samples were allowed to clot for 24 h at 4°C, then were centrifuged (1,935×g for 15 min), and serum was harvested and stored at -20°C until assayed for progesterone using a solid-phase, no-extraction radioimmunoassay.

#### Ultrasonography

Ultrasound examination of the ovaries was performed, using a Chison 600VET Ultrasound (Japan) equipped with a linear array 5 MHz transrectal transducer, to determine ovulatory response to Ovsynch and post-insemination treatments. Ultrasound examinations were performed at day -10 and at day 0 and day 1 after AI to confirm ovulation. Cows were considered to have synchronized ovulation after the second GnRH injection of Ovsynch when 1 or more follicles ≥10 mm were present at AI and were absent at an ultrasound examination

conducted 2 days later. Ultrasound examinations were continued every 3 days from day 5 to day 23 after AI (Fig 1). Pregnancy status was determined at day 45 after AI by transrectal ultrasonography.

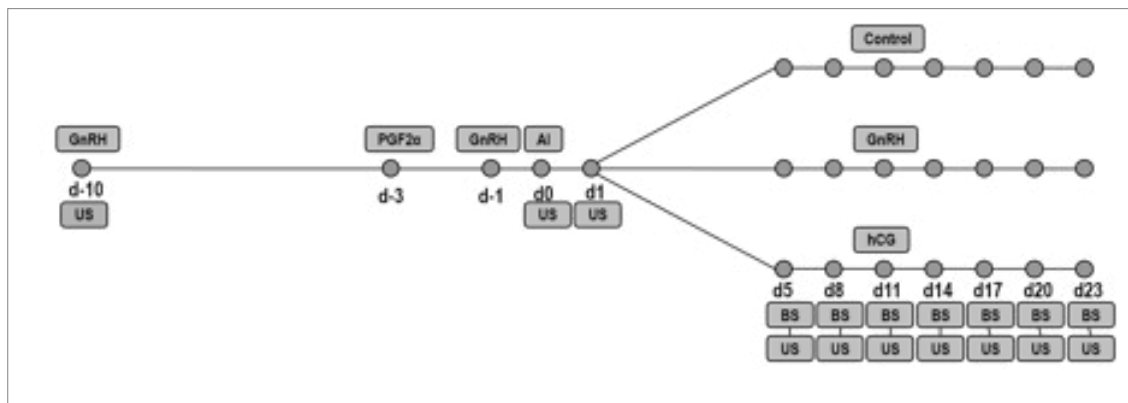
#### Statistical analysis

Because progesterone and CL area were measured over time, a repeated measures approach using ANOVA with mixed linear models (fixed effects of treatment, day and their interaction, random effect of cow) was used in SAS (Version 9.1.3; SAS Institute Inc., Cary, NC). For clarity, corresponding means ± SEM of the non-transformed data are presented in the results. Additionally, a mixed general linear model was fitted using the MIXED procedure of SAS (random effect of cow) to evaluate the effect of treatment on pregnancy rate. A Pearson correlation coefficient between progesterone concentrations and total area of luteal tissue and between dominant follicle diameter on day 11 and accessory CL area at day 17 after AI were calculated by the PROC CORR procedure in SAS.

## RESULTS

#### Pregnancy rate

The pregnancy rate was 45.45% (5/11) in either control or GnRH-treated cows. However, this rate was 54.54% (6/11) in hCG-treated cows. There was no significant difference ( $p > 0.05$ ) between groups.

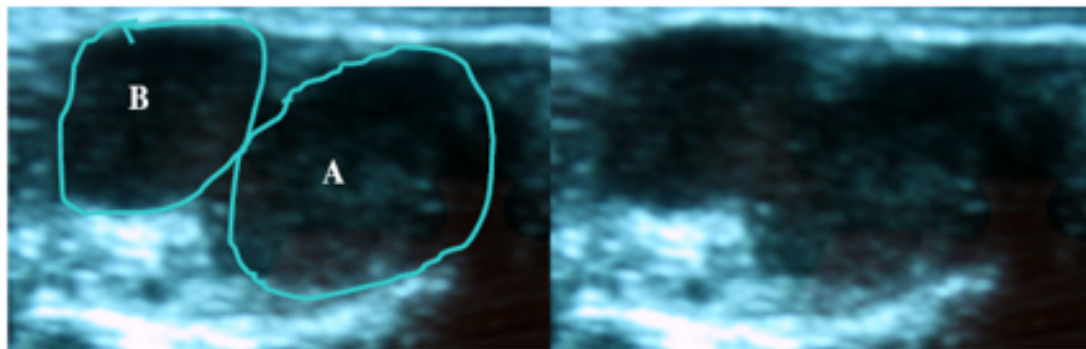


**Fig 1:** Schematic diagram of activities and treatment schedules of control group (Saline), day 11 GnRH group (gonadoreline 100 µg) day 11 hCG group (hCG, 1500 IU) in cows (AI: artificial insemination; BS: blood sample d: day; PG: prostaglandin; US: ultrasonography).

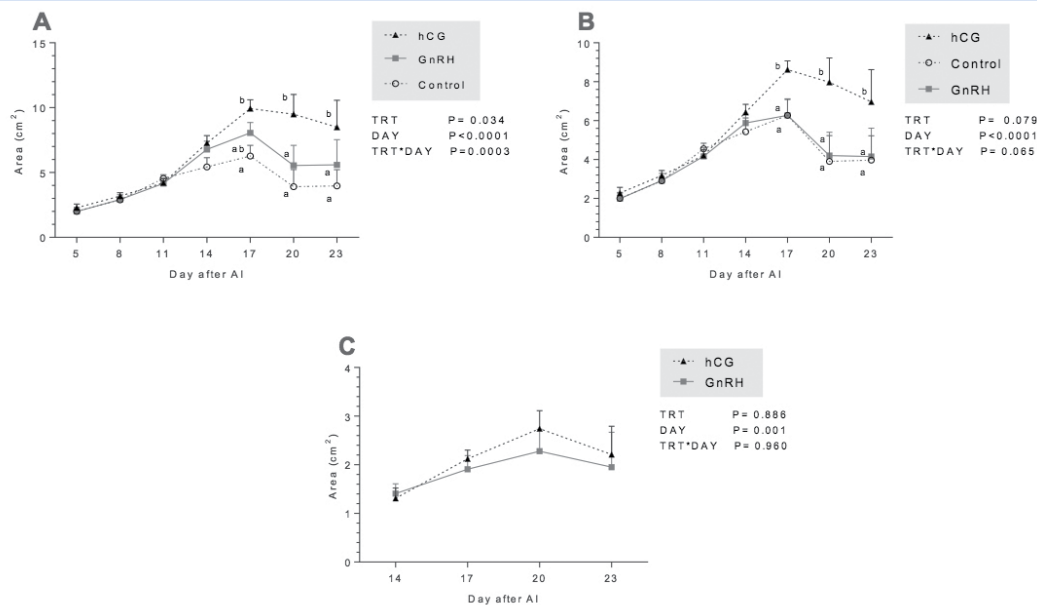
### Ovarian status of cows at day 11 after AI

The ultrasound examination at day 11 after AI (the day of GnRH or hCG injection to induce ovulation or luteinisation of the dominant follicle) revealed the presence of one CL (no double ovulations were detected after AI) and one dominant follicle. The mean diameter of the dominant follicle on day 11 after AI was  $11.74 \pm 1.34$ ,  $12.06 \pm 1.31$  and  $11.86 \pm 1.31$  mm in the hCG group, GnRH group and control group, respectively. In cows treated with GnRH or hCG, the mean ( $\pm$  S.E.M.)

diameter of the dominant follicle for cows that subsequently formed an accessory CL (14/22) was  $14.52 \pm 0.61$  mm (maximum diameter: 18.6 mm, minimum diameter: 10 mm). The mean follicular diameter for cows that have not formed an accessory CL (8/22) was  $7.28 \pm 0.4$  mm (maximum diameter: 9 mm, minimum diameter: 5 mm). There was a significant difference in the diameter of dominant follicle between cows that have formed an accessory CL and cows that have not formed an accessory CL ( $p=0.004$ ).



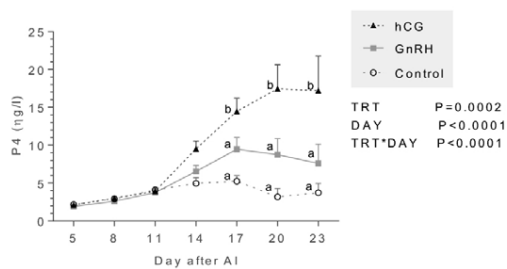
**Fig 2:** Sonogram image of an ovary at day 24 post AI showing two CL after GnRH treatment: (A) principal CL, (B) GnRH-induced accessory CL



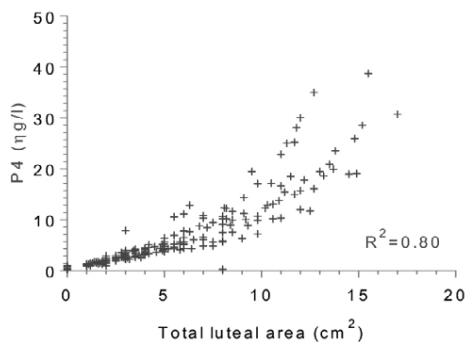
**Fig 3:** Mean ( $\pm$  SEM) of area of luteal tissue (cm<sup>2</sup>) in control cows that did not receive treatment, in cows that received 1500 IU of human chorionic gonadotrophin (hCG) and in cows treated with GnRH. (A) Mean ( $\pm$  SEM) of total area of luteal tissue (accessory CL+ principal CL). (B) Mean ( $\pm$  SEM) of principal CL area. (C) Mean ( $\pm$  SEM) of accessory CL area. (TRT: type of treatment, DAY: day of measurement, TRT\*DAY: interaction treatment by day).

### Corpora lutea

Treatment at day 11 induced ovulation of the dominant follicle and formation of an accessory CL in 63.63% (7/11) of cows treated with GnRH and in 63.63% (7/11) of cows treated with hCG. However, none of the control cows formed an accessory CL (Fig 2). Thus, there was an acute disappearance of the dominant follicle and subsequent appearance of an accessory CL at the same location on the ovary in GnRH and hCG treated cows. Total CL areas was significantly higher at day 20 and 23 in hCG-treated cows compared to GnRH and control groups (Fig 3.A). Furthermore, principal CL areas was higher at day 17, 20 and 23 in hCG group compared to GnRH and control group (Fig 3B). There were significant effects of measurement time on total and principal CL areas ( $p < 0.0001$ ) (Fig 3. A and B), and there was an effect of group-by time interaction ( $P = 0.0003$ ) for total CL area (Fig. 3A). However, the



**Fig 4:** Mean ( $\pm$  SEM) of progesterone concentration in cows (ng/mL) .



**Fig 5.** Correlation between progesterone concentrations and total area of luteal tissue. Both principal and accessory CLs were taken into account

accessory CL area induced by GnRH and hCG showed no significant change through the time and no group-by time interaction was observed (Fig 3. C). A significant correlation ( $r = 0.83$ ,  $p < 0.001$ ) was found between dominant follicle diameter on day 11 and accessory CL area at day 17 after AI.

### Plasma concentration of progesterone

Cows treated with hCG presented significantly increased circulating progesterone concentrations from day 17 to day 23 after AI relative to the control and GnRH groups (Fig 4). There was an effect of group-by time interaction ( $p < 0.0001$ ). A significant correlation ( $r = 0.89$ ,  $p < 0.001$ ) was found between progesterone level and total CL area (principal CL + accessory CL) for each day (Fig 5).

### DISCUSSION

Previous studies showed that administration of GnRH, a GnRH agonist or hCG after AI at specific times coincident with the presence of the dominant follicle of the first and second follicular waves may stimulate CL function, induce accessory CL formation, increase progesterone concentration and reduce oestrogen production with a consequent positive effect on embryo survival (Stevenson et al., 2007).

In the present study, the diameter of the dominant follicle was higher in cows that formed an accessory CL compared to those that did not ( $p = 0.004$ ). Similarly to our results, Musilová et al (2014) observed that the incidence of two CL was higher in cows bearing the largest follicles from 10 to 20 mm in diameter compared to cows bearing only follicles  $\leq 9$  mm. Whether cows respond to GnRH may depend upon the characteristics of follicular growth; a follicle must reach 10 mm in diameter to ovulate in response to LH (Sartori et al., 2001). In the current study, 63.63% ( $n = 14/22$ ) cows treated with hCG or GnRH at day 11 after AI had formed an accessory CL; these results were higher than that (33.5%) reported by Musilová et al (2014) following a lot on the fact that others have used different dose of hCG? GnRH injection on day 11-13. Stevenson et al (2007) found an almost similar (60% for GnRH-treated cow) or greater (77.5% for hCG-treated cows) accessory CL formation after treatment of the cows between day 4 and day 9 after AI. This finding may be explained by the timing of treatment.



The type of hormone (hCG or GnRH) injected at day 11 after AI did not really influence the size of the accessory CL ( $p=0.88$ ). However, the principal CL area was significantly larger in cows treated with hCG compared to that of cows treated with GnRH and the control cows. There was no significant difference between the area of principal CL in cows treated with GnRH and control cows. Despite difference in doses, our results match with those found by Stevenson et al (2007), Stevenson and Pulley (2012) and Rizos et al (2012). Previous studies in dairy cows have reported similar frequencies of newly formed luteal structures when hCG was administered post AI at doses ranging from 1,000 to 3,300 IU (Santos et al., 2001; Stevenson et al., 2007). The increase of luteal tissue area arises from the LH-like action of hCG on luteal cells (Schmitt et al., 1996a). In the present study, administration of hCG not only resulted in ovulation of the dominant follicle present on day 11 and the formation of an accessory CL but also stimulated the principal CL in treated cows, leading to an increase in the area of luteal tissue in that CL, in addition to an increase in luteal tissue associated with the presence of the accessory CL.

The main objective of administration of GnRH or hCG is to improve the fertility of post-partum dairy cows by increasing progesterone concentration (Santos et al., 2001; Stevenson et al., 2007; Rizos et al., 2012; Maillo et al., 2014). In the present study, injection of hCG on day 11 increased plasma concentrations of progesterone compared with that of controls or GnRH treated cows. Several authors (Franco et al., 2006; Cruz et al., 2009, Ataman et al., 2011; Musilová et al., 2014) detected higher progesterone concentrations in dairy cows after induction of an accessory CL with GnRH or hCG administered between day 11 and 15 after AI. It is worth mentioning that the stimulation of progesterone secretion by the principal CL after treatment with hCG was higher compared with that of GnRH. This finding is supported by the observation of an increase of principal CL area in hCG- treated cows compared with GnRH-treated cows with a strong positive and close correlation between the total luteal area and the progesterone level in the current study ( $r=0.89$ ,  $p<0.0001$ ) and in previous studies (Kastelic et al., 1990; Herzogn et al., 2010) between the total luteal area and the progesterone level. Indeed, Mann (2009) showed that progesterone level is related to the diameter of the CL that secretes

progesterone until it reaches its final size.

The longer half-life of hCG in the blood and the slower turnover of the hCG-LH receptor complex on the surface of granulosa cells are probably responsible for an increased gonadotropic stimulation on the day 5 ovulatory follicle (De Rensis et al., 2010; Lonergan 2011) and its subsequent differentiation into a principal CL with a greater progesterone secreting capacity. The half-life and therefore the LH-like effect of hCG on the ovarian cells may last for 30 h after treatment (Schmitt et al., 1996a); in contrast, administration of 10mg buserelin increases LH concentrations in serum for approximately 5 h (Chenault et al., 1990). Moreover, the CL formed after ovulation induced by GnRH may not be fully functional (Santos et al., 2001; Schmitt et al., 1996b). Thus, hCG treatment usually elevates progesterone concentrations more than GnRH treatment does (Schmitt et al., 1996a; Stevenson et al., 2007).

It has been suggested that the increase in progesterone after hCG administration is due to the progesterone produced by the induced CL. In the study of Schmitt et al (1996b), plasma progesterone did not differ between the control and hCG groups after removal of the accessory CL on day 13. Thus, other evidence indicates that the increased progesterone cannot be entirely attributed to the induced CL. For example, administration of hCG leads to an increase in the area of luteal tissue in the principal CL in addition to the increase in total luteal tissue associated with the presence of the accessory CL (Rizos et al., 2012).

In conclusion, the injection of 1500 IU hCG or 100  $\mu$ g GnRH in dairy cows at day 11 after AI results in the formation of an accessory CL, in cows with a dominant follicle with a diameter  $\geq 10$  mm. Compared with the control group, only hCG-treated cows had increased progesterone production.

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#### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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