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Effect of Estradiol Benzoate on the Size of Follicle and Corpus Luteum of Holstein Cows Treated with D-Cloprostenol

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ABSTRACT

The effect of two treatments, farms, and body shape on the size of the follicle and Corpus Luteum were evaluated. D-Cloprostenol (150 ug) was used in one of the treatments; in the other, D-Cloprostenol (150 ug) and Estradiol Benzoate (1 mg), were used. Two groups of 32 Holstein dairy cows each, from the Nero and Irquis farms in Cuenca, Ecuador, were set up. Body condition (2.75-3.50) was considered. The calving number was 1-6; the farms, Irquis and Nero; and animal age, 3-10 years. Ultrasound scanning was used on days 0; 3 and 7, in order to measure the Luteum first, then the dominant ovarian follicle, and finally, the Corpus Luteum. Factor analysis of variance was performed. No significant differences were found for follicle size due to any factors; nor due to interaction. Concerning luteum, significant differences ($P < 0.05$) were observed for the treatments and for the farm. The values were 0.33 cm higher for the treatment with Benzoate, and 0.36 cm, at Irquis, in comparison to Nero. The treatment with Benzoate, along with better husbandry at Irquis played a critical role in the appearance and evolution of the luteum.

Key words: *body condition, hormones, estrus cycle, reproduction*

INTRODUCTION

High performance cows are known to have a different endocrinological status from non-lactating dairy cows, due to their metabolic rate. Cows with elevated production of milk can develop bigger follicles with a lower concentration of circulating estradiol (Walsh *et al*., 2011; Satheshkumar *et al*., 2015; Szelenyi *et al*., 2015). Moreover, highly productive cows have greater volume of luteal tissue and lower concentration of circulating progesterone. The most reasonable explanation indicates that ovulation of an overstimulated prematurely activated ovocyte may take place, with ensuing reduction in fertility (Pastzinca and Molina, 2007; Walsh *et al*., 2011; Rodríguez *et al*., 2012).

Transrectal ultrasound used to view follicular development and the early presence and growth of corpus luteum, is one of the most practical applications of dairy cattle reproduction. Early identification of these problems can improve the productive efficiency and the gestation percent through corrective actions.

The use of exogenous estradiol along with progesterone, leads to suppression, or decrease in the main follicle diameter, when applied before or during the wave, seemingly due to FSH and possibly LH surges. When follicle selection is done, the treatment results in a decrease of the dominant follicle diameter (McDougal *et al*., 2004). Likewise, other progestin and estradiol combinations have been used with variable results, plus the possible adverse effects caused by estradiol used after artificial insemination (AI) on the corpus luteum, which calls for more research (Pastzinca and Molina, 2007; Bertot *et al*., 2012; Boldt *et al*., 2015).

In that sense, the aim of this paper is to assess the effect of benzoate estradiol treatment, the farm and the temporary body condition of Holstein cows synchronized with D-Cloroprostenol on follicle size and corpus luteum.

MATERIALS AND METHODS

The research took place on the Nero and Irquis farms, at the Faculty of Agricultural Sciences of the University of Cuenca, Province of Azuay, Republic of Ecuador, located at 02° 55' 00'' and 03° 04' 00'' south latitude, and 79° 03' 00'' and 79° 05' 32'' west longitude, respectively, 2 500 meters above sea level, with high tropical climate, and mean annual temperatures of 10° C, 1 500 mm annual rainfall, and well distinctive rainy and dry seasons (Weather Station, University of Cuenca).

A completely random design with factorial arrangement was used in an experiment with 64 Holstein cows; 32 per farm, between 3-10 years of age, and 2-6 parturitions. Their body conditions were assessed in a 5 point scale, and the resulting values (2.75 and 3.50) were taken as variation sources along with the farms. Irquis and Nero had an average of 17.2 and 15.6 kg/cow/day of milk production, with lactation periods between 92 and 116 days, respectively. Two treatments were used: D-Cloroprostenol (150 ug), identified as PGF and D-Cloroprostenol + Estradiol Benzoate for injection (1 ml/cow, 24 h following D-Cloroprostenol), identified as PGF+EB to measure its effect on follicle size and corpus luteum on days 0; 3 and 7 after, with four observations per treatment.

Each animal´s record was checked in the two farms, in order to assess their productive and physiological condition. The cows received physical and clinical checkup. Measurements were made through ultrasound scanner (Aloka 505) with a 5.5 Mb probe. The different stage images were saved, and the follicles and corpus luteum were measured (cm) through echography. Factorial variance analysis and the Duncan test (1955) were made for significance, using SPSS 11.0.

RESULTS AND DISCUSSION

Pastzinca and Molina (2007) pointed out that high performance dairy cows may have a different endocrine status from non-lactating cows, due to their high metabolic rate. The cows with higher milk production develop bigger follicles, but with lower circulating estradiol concentration. In addition to it, these particular cows have more luteal tissue volume and lower concentrations of circulating progesterone. The most reasonable explanation is that ovulation of an overstimulated prematurely activated ovocyte may take place, with ensuing reduction in fertility (López *et al*., 2004; Wiltbank *et al*., 2006; Rodríguez *et al*., 2012; Szelenyi *et al*., 2015).

Follicle growth and development in ruminants are characterized by two or three consecutive follicular waves per estrus cycle. According to reviews by Pastzinca and Molina (2007), and experimental works like Satheshkumar *et al*. (2015) and Szelenyi *et al*. (2015), ultrasound techniques have contributed to collecting a lot of information about the stages of growth and follicle selection under different management and location circumstances. Each wave means recruiting new cohorts in the ovary reserves, and allows for the selection of a dominant follicle that grows and matures, until the pre-ovulation stage is reached.

In that sense, the results of the PGF and PGF + EB treatment effects indicated no significant differences regarding follicle size (Table 1). It is related to the very same situation previously stated, in which under cascading conditions of high levels of estradiol follicle sizes were affected, and as in studies by Wiltbank *et al*. (2006) and Boldt *et al*. (2015) of high performance dairy cows. Moreover, lower concentrations of circulating estradiol were observed, which leads to a short and less intense estrus.

In the same way, the effect of farms on follicle size was not significant (Table 2), which implies that the estradiol levels applied to the treated animals in each case, were compensated by the greater immature follicle, even in untreated animals, also reported in various works on cows with high milk production (Wiltbank *et al.,* 2006; Walsh *et al*., 2011; Parr *et al.,* 2015).

Reduction in estradiol concentration causes a decrease in the duration and intensity of estrus (Boltd *et al*., 2015). Accordingly, such reduction might also cause an increase in follicle size due to prolongation of the interval previous to estradiol-induced estrus, the GnRH-LH peak, and ovulation of highly producing cows, which can explain the absence of differences between treatments for this indicator of farm follicle morphology on the farms, each with high production potential dairy animals.

The differences in body conditions were insignificant for follicle development and morphology (Table 3). Changes in the body condition status are known to cause reductions or increases of 30-55 kg of life weight to change a unit condition also imply nutritional effects at the ovary level, in terms of glucose, lipid, amino acid, and mineral supplies, among others, for growth and development (Rodríguez *et al*., 2012; Parr *et al*., 2015).

Global dairy cow fertility decreased in the last five decades, as a consequence of the potential and actual production of milk. In that sense, Walsh *et al*. (2011) stated several hypotheses that explain variations of follicle development through genetics, physiology, nutrition, and the physical condition of the animal. On many occasions, they were unable to define the cause of such variations through body condition, which was not very distinctive in terms of follicle and luteal development. It was corroborated in other studies with high potential cows with energy unbalance, very similar body conditions, and prolonged nutritional deficiencies (Thatcher *et al*., 2002; García López, 2003; Rodríguez *et al*., 2012; Parr *et al*., 2015).

Likewise, Satheshkumar *et al*. (2015) reported a significant reduction in luteal activity in the warmest months of the year, and it was associated to a lengthy phase of follicle growth that altered endocrine activity of LH, and caused lower fertility values in herds with Jersey-Zebu cows, in different body conditions and nutritional states.

A different behavior was determined in the morphometrics of the corpus luteum, when the treatments applied were compared (Table 4), and the treatments with estradiol benzoate was significantly higher ($P < 0.05$) with a mean value of 1. 981 cm, compared to 1. 763 cm of PGF alone.

Recent studies have proposed systems through which an injection of estradiol was replaced with the administration of GnRH at the beginning of treatment (Walsh *et al*., 2011; Moussa *et al*., 2015; Parr *et al*., 2015). This change is clearly associated with the European ban on estradiol esters in food producing animals. One of the recent modifications to the systems with synchronization, based on progestins consists in the administration of a low dose (0.5-1.0 mg) of estradiol benzoate, 24 h after progestin withdrawal.

This strategy for treatment application also increased the accuracy of estrus start, and triggered estrus signs, which facilitated estrus detection. The exogenous estradiol is also expected to exert a tighter accuracy control when the LH peak, luteal development and ovulation are produced. It coincides with other works where the same variant was applied by Walsh *et al*. (2011) in high producing stable dairy cows, and another by Szelenyi *et al*. (2015).

The significant differences found between the farms for the corpus luteum (Table 5) are related to a better nutritional and management status at Irquis, in comparison to Nero. The former shows higher dairy production per lactating cow per day, which implies a better nutritional and body status of animals. This issue has effects on the reproduction indexes, as well as on the animals' willingness to express every favorable sign in the estrus cycle. As animals ready for a new gestation, they must continue the important cycle for the dairy system, indicated by Ugarte (1995) and García López (2003) of calving, lactating, gestating and again, calving.

The experiment results coincide with studies by Parr *et al*. (2015), who found problems in follicle development and luteal formation in negative periods of energy balance, experienced by post-calving cows, which led to undesirable changes in their metabolism. These make them incompetent for adequate formation of follicles, and even gestation. This issue is also linked to a loss in body condition in animals with more nutritional restrictions, as may have been the case of the Nero cows at that time, which had a lower performance than Irquis' (Narváez, 2015), personal communication).

Estradiol use at the onset of a synchronization treatment with progesterone, even when its duration is up to 12 days, not always guarantees complete regression of the corpus luteum in all the animals at the time progesterone is withdrawn, or 24 h later. As a consequence, $PGF_{2\alpha}$ should be administered when progesterone is withdrawn, or some hours before, to ensure the regression of the luteal body in non-responding animals to estradiol.

The benzoate protocol implies the replacement of the second GnRH shot by estradiol esters. Stevenson *et al*. (2004), Bertot *et al*. (2012); Pastzinca and Molina (2007), and Walsh *et al*. (2011) indicate that estradiol synchronizes ovulation of the dominant follicle within a narrower time lapse, and it also increases the expression of estrus behavior in cows treated to shorten the life of the corpus luteum and induce the appearance of a new follicle. However, it cannot entirely block LH secretion, and a small pulse secretion is kept, which allows the presence of a dominant follicle, whether there is one at the onset of treatment.

CONCLUSIONS

Important effects were observed on the size of the corpus luteum, related to estradiol treatment factors that increased the action of the estrogenic compound, and differences were observed at the farm level, due to the animals´ productive condition.

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Table 1. Effect of treatment with PGF and PGF+BE on follicle size (cm) in cows from the two farms

Treatments	Mean	Typical error	Significance (P < 0.05)	Lower cut off	Upper cut off
PGF	1.889	0.0591	NS	1.771	2.007
PGF+BE	1.965	0.0561	NS	1.851	2.078

Table 2. Effect of farm on follicle size (cm) in the treated cows

Farm	Mean	Typical error	Significance (P<0.05)	Lower cut uff	Upper cut off
Irquis	1.929	0.0571	NS	1.813	2.044
Nero	1.925	0.0562	NS	1.809	2.041

Table 3. Effect of estimated body condition on follicle size (cm) in cows from the two farms

Body condition	Mean	Typical error	Significance (P<0.05)	Lower cut off	Upper cut off
2.75	1.967	0.0802	NS	1.807	2.127
3.00	1.997	0.0805	NS	1.837	2.158
3.25	1.876	0.0801	NS	1.716	2.036
3.50	1.867	0.0862	NS	1.694	2.040

Table 4. Effect of treatment with PGF and PGF + BE on lutheal body size (cm) in cows from the two farms

Treatments	Mean	Typical error	Significance (P < 0,05)	Lower cut off	Upper cut off
PGF	1.763	0.0271	*	1.602	1.882
PGF+BE	1.981	0.0146	*	1.808	2.115

Table 5. Effect of farm on lutheal body size (cm) in the treated cows

Farm	Mean	Typical error	Significance (P<0.05)	Lower cut off	Upper cut off
Irquis	1.978	0.0104	*	1.561	2.194
Nero	1.612	0.0112	*	1.309	1.901