



## CUT-OFF POINT OF GLUTATHIONE PEROXIDASE(GSH-PX) FOR THE DIAGNOSIS OF ENDOMETRITIS IN DAIRY COWS

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**ABSTRACT:** *The cut-off point of a diagnostic test is the determination of the minimum value at which an individual can be diagnosed as sick. To obtain a good position, an analysis is carried out using a statistical ROC Curve (Receiver Operating Characteristic), which is used in determining the value of the diagnosis with the highest sensitivity and specificity. The cut-off value of glutathione peroxidase (GSH-Px) for the diagnosis of uterine diseases is not described. The minimum tolerable value can be described for 130 U/g Hb; the value has been used as a limit under which it has a marginal deficiency of Se. Therefore, the objective of this work was to determine the cut-off point of the GSH-Px values that allows diagnosing cows with endometritis, in dairy herds in the province of Santa Fe, Argentina. We used 115 Holstein cows between 21 and 56 postpartum days, from dairy establishments in the province of Santa Fe, Argentina. Blood samples were obtained by puncturing the coccygeal vein for determination of GSH-Px. At the same time, the uterine status of the cows was characterized in two groups: healthy cows (VS, by its initials in Spanish) and cows with endometritis, either clinical and subclinical (VE, by its initials in Spanish). To create a ROC curve, the VS and VE diagnoses must be entered under a binary system as 0 (VS) and 1 (VE). The obtained results indicate that the point or cut value for the GSH-Px was of 222 U/g Hb with a sensitivity of 81.4% and a specificity of 33.9%. That is to say, above that value, the possibility of suffering endometritis decreases significantly. We can conclude that the cut-off point of 222 U/g Hb obtained for this group of cows from establishments in the province of Santa Fe, could be considered in the future as a complementary tool to diagnose endometritis.*

**KEYWORDS:** Glutathione peroxidase, Dairy cows, Cut-off point.



## INTRODUCTION

Selenium (Se) is a trace element that plays an important role in the performance and health of animals. In dairy herds, Se deficiency may have economically significant impacts, such as fertility reduction, placental retention, and the incidence of mastitis and metritis (Spears et al., 2008; Eulogio et al., 2012). By decreasing Se concentration in the diet, the activity of the glutathione peroxidase enzyme (GSH-Px) is reduced. This is directly related to a greater predisposition to undergo oxidative stress (Ceballos et al., 1998). GSH-Px is a seleno-dependent enzyme whose function is to catalyze the reduction of peroxides by eradicating free radicals and thus protecting the organism of tissular damage (Sandholm, 1980). Se can be found in the structure of GSH-Px and the activity of the enzyme can be directly correlated with blood/tissular concentration of Se (Thompson et al., 1980). Organic alterations, related to oxidative disturbances, are more important in animals with Se deficiency in their diet, whether they are related or not to low concentrations of vitamin E, and especially in situations in which there is an intense metabolic activity that overwhelms the cellular defense mechanisms and causes numerous toxic effects to appear.

The intensification of production systems together with genetic selection in animals has increased animal production. Alongside, the imposition of greater metabolic demands in animals has predisposed them to develop the so-called production diseases (Wittwer, 2012). Thus, 75% of the incidence of diseases, such as mastitis, metritis, abomasal torsion, and ketosis occurs in the first month of lactation (Le Blanc et al., 2006). The correct and early diagnosis of present uterine pathologies is of great importance since it would allow to act in time and to maintain the reproductive efficiency of the heard (De la Sota et al., 2014). The cut-off point of a diagnostic test consists in setting a minimum value above which an individual can be diagnosed as sick. There is no cut-off point of Se levels, nor for GSH-Px values, to diagnose clinical and subclinical endometritis, diseases that lead to a real decrease in the reproductive efficiency, increasing the calving-conception interval (Rinaudo et al., 2017).

The objective of this study was to set the cut-off point of the GSH-Px values that allows diagnosing cows with endometritis in dairy herds in grazing systems.

## MATERIALS AND METHODS

We used 115 Holstein cows between 21 and 56 postpartum days, from commercial dairy establishments in Centeno, 32° 18'00 "S 61° 25'00" W, a town located in the province of Santa Fe, Argentina. Blood samples were obtained by puncturing the coccygeal vein for the determination of GSH-Px. After each extraction, the blood was placed in a tube with anticoagulant (heparin), centrifuged and the plasma taken. The samples were kept in Eppendorf tubes at -20°C until analysis. Se concentration was evaluated through the activity of the GSH-Px. The determination of its activity, in all cows, was through the plasma with a Randox Kit (RANSEL) in Laboratorio Azul, province of Buenos Aires. The uterine status of the cows was characterized in two groups: healthy cows (VS) and cows with endometritis either clinical or subclinical (VE), while analyzing the presence of clinical and subclinical endometritis.

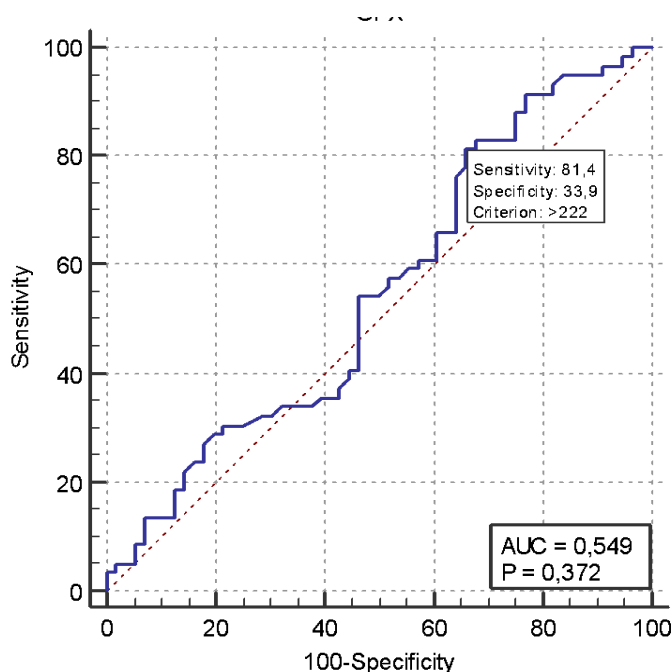
Clinical endometritis was diagnosed according to the presence of mucopurulent cervicovaginal discharge. Such discharge was obtained through a manual technique, which consists in the



introduction of the glowed arm through the vagina, extraction of the discharge and direct observation. Those discharges that had pus were classified as positive for clinical endometritis and these cows were not included in the diagnostic of subclinical endometritis (Sheldon et al., 2006). Subclinical endometritis was diagnosed using the complementary diagnostic technique known as Cytobrush. Cytological preparations were observed with an optical microscope at an increase of 400X and a minimum of 200 total cells (epithelial cells and inflammatory cells) were counted, from which the proportion of inflammatory cells (polymorphonuclear neutrophils) was determined. To determine the degree of inflammation of the uterine mucosa, the percentage of polymorphonuclear neutrophils was calculated. The cut-off point to determine the presence of SE used was that of  $\geq 5\%$  polymorphonuclear neutrophils (Rinaudo et al., 2012). A cow without the presence of clinical and SE which got pregnant within the 210 postpartum days was considered healthy, and a cow that did not get pregnant before that period was considered sick or with clinical and subclinical endometritis. For the analysis of the ROC curve, the statistical program MedCalc 12.2.1 was used in its free trial version. In this program, the diagnoses of healthy cows or sick cows must be entered under the binary system as 0 (VS) or 1 (VE).

## RESULTS

The results obtained indicated that the point or cut for the GSH-Px was 222 U / g Hb with a sensitivity of 81.4% and a specificity of 33.9%. That is to say, above that value, the possibility of suffering endometritis decreases significantly and the chances of achieving pregnancy before 210 days increase.





## DISCUSSION

The transition period has been defined as the most critical period related to the health of dairy cows and during the lactation cycle.

In fact, 75% of the diseases in dairy cows occur within the first month of lactation (LeBlanc et al., 2006). The transition of dairy cows shows dramatic changes in energy balance, alteration of the immune system, and oxidant / antioxidant balance (Leblanc, 2010; Sundrum, 2015). These alterations can affect the health, fertility, and longevity of dairy cows (Lutoslawska et al., 2003; Elischer et al., 2015). The result obtained from the limit point of the GSH-Px value was 222 U/g Hb, higher than that reported by Ceballos et al. (1998b), in which the minimum tolerable is 130 U/g Hb; value that has been considered as the limit under which there would be a marginal deficiency of Se. Cows could develop metabolic stress if they fail to reach the minimum levels of U/g Hb displaying a greater presence of clinical and subclinical endometritis.

## CONCLUSION

It is concluded that the cut-off point of 222 U/g Hb obtained from the group of dairy cows studied from establishments in grazing systems could be considered in the future as a complementary tool to diagnose endometritis.

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