

Evaluation of tear secretion by phenol red thread test in Holstein cows

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Summary

This study aimed to measure the normal physiological tear production of Holstein cows with the phenol red thread (PRT) test to determine the reference value specific to this test and to examine the effect of the number of births, age and seasonal air temperature on the amount of tear production. The study was performed on 50 Holstein cows aged 3-7 years in two groups. The PRT test was performed in group 1 in January and in group 2 at the end of May. The age and birth numbers of all animals were recorded. The mean amount of tear secretion for all Holstein cows ($n = 50$) was 32.90 ± 0.73 mm/15 sec. The mean value was 34.47 ± 1.28 mm/15 sec. for group 1 ($n = 23$) and 31.55 ± 0.73 for group 2 ($n = 27$). When the seasonal temperature increased from 12°C to 20°C, the difference in the mean amount of tear production between group 1 and group 2 was statistically significant ($P < 0.05$). Although there was a tendency for the amount of tears produced to increase with the increase in the number of births, it was not statistically significant ($P > 0.05$). Similarly, the decrease in the number of tears with increasing age was not statistically significant ($P > 0.05$). This study determined reference values for tear secretion in healthy Holstein cows with the PRT test. Higher PRT scores were recorded in winter than in spring ($P < 0.05$), and the change in PRT values was not significant as the number of births and age increased ($P > 0.05$).

Keywords: cow, tear secretion

Dry eye, xerophthalmia or keratoconjunctivitis sicca (KCS), is defined as a progressive inflammatory condition of the cornea and conjunctiva resulting from decreased lacrimation (25). Autoimmune illnesses, medications, radiation therapy, orbital or supraorbital trauma, ocular surgery, and neoplasia are among the ophthalmic causes of KCS, while systemic causes include neurogenic, metabolic or infectious diseases and systemic drugs (24). Although not common, it has been reported that keratoconjunctivitis sicca, glaucoma (19, 29) and some herbal poisonings (locoweed, thorn apple, rapeseed) are among diseases that cause a decrease in the amount of tear production in cattle (17, 24). The effects of hormones and aging on the development of KCS have been investigated. It has been reported that the deficiency of androgens causes atrophy in the lacrimal gland, affecting the size and function of the gland and autoimmunity, and prolactin is an endogenous tear suppressor (3, 4, 25). Moreover, the incidence of KCS increases with increasing age in humans (8) and dogs (4).

Dry eye is thought to be affected by the humidity of the living environment, which might alter due to low humidity in winter and high evaporation in summer (24). It has been observed that the amount of aqueous tear production in animals is affected by their breed and that it varies depending on the breed even when the same tests are applied (23, 28). According to the etiopathogenesis, dry eye is classified as dry eye with a lack of watery tears or dry eye caused by evaporation (due to internal or external causes). It has been reported that the diagnosis of dry eye is usually difficult and complex because of its multifactorial nature in species with unknown normal test ranges (21, 30).

Diagnosis of KCS is based on clinical findings, laboratory analysis and Schirmer tear test (STT) results. However, clinical misdiagnosis of KCS may lead to severe eye damage (2). While comprehensive ophthalmic and physical examinations are required to identify the underlying cause of KCS, blood tests or other additional procedures may be necessary in some cases to formulate a definitive diagnosis (24).

Some tests are used to determine the amount of tear secretion, which decreases in animals with dry eyes. The Schirmer tear test (STT) is the most extensively used of these tests (9, 20). The Schirmer test has some disadvantages, such as varied normal values for different races, a long application time and eye irritation due to the large test paper. Because of these disadvantages, the phenol red thread test (PRT) has been developed as an alternative to STT (7). The PRT test uses a 75 mm long thread with a 3 mm twist at one end impregnated with phenol red, a pH indicator. The thread is placed in the lower conjunctival fornix for 15 seconds. When soaked with alkaline tears, the pale-yellow thread turns orange. The amount of tear production is determined by the length of the orange wetness measured on a millimetre scale (14, 28).

The PRT test has been used to determine typical PRT readings in a variety of domestic and wild animal species and breeds, as well as in humans (2, 7, 16, 22, 23, 26). The PRT test has the advantage of stimulating rapid (15 seconds), painless and low-grade reflex tears (14, 28).

In the literature, there are a few studies on STT measuring tear production in Holstein cows (32, 37), but none on PRT. The goal of this study was to use the PRT test to determine the test's unique reference value by measuring the normal physiological tear production of widely reared Holstein cows and examining the effect of the number of births, age, and seasonal air temperature on the amount of tear production.

Material and methods

The study was conducted on 50 Holstein cows aged 3-7 years. The cows were clinically healthy and did not have any eye disease in the past. All tests were performed in a random eye for each animal. Previous studies on this subject have shown that there is no difference between the right and left eye in the amount of tears produced (23, 35). The tests were carried out in a semi-open barn on the farm. The animals were restrained by an assistant at the chest level. No sedatives or anaesthetics were used in the study. All tests were performed by the same investigator between 12:00 and 15:00 during the day.

Two groups of random cows were formed: group 1 ($n = 23$), in which the PRT test was performed in January at $+12^{\circ}\text{C}$ air temperature, and group 2 ($n = 27$), in which the PRT test was performed in May at $+20^{\circ}\text{C}$. Birth numbers of all animals were recorded.

Sterile cotton threads (Zone-Quick; AYUMI Pharmaceutical Corp., Tokyo, Japan) impregnated with phenol red of 75 mm length with 3 mm folded ends were used for measurement. During the measurement, the eyelids of each cow were opened with fingers, and the PRT test thread was placed in the conjunctival lateral fornix at approximately one third of the lower eyelid, and the eyelids were closed (Fig. 1). The thread was removed after being left to soak tears for 15 seconds. The yellow colour of the phenol red-impregnated yarn and the wet area, which is turned orange



Fig. 1. Placement of the PRT thread test

red by the slightly alkaline tears, were immediately measured with a millimetric ruler on the package containing the yarns.

Descriptive statistics were calculated as the mean amount of tear production within groups and the standard deviation from the mean in the study. The differences between the means of the groups and within the groups were analysed by the least squares' method, and the differences between the group means were determined. The relationships between the number of births and the amount of tear production were analysed with Spearman's correlation. All calculations and analyses in the study were performed with the SAS (2009) statistical package program. The relationship between air temperature and the age of the cows used in the study and PRT tear measurements was determined linearly by normal regression analysis. The study aimed to identify the effects of age and temperature, which are accepted as independent variables in the ordinary regression model, and the effects of one-unit changes in age and temperature on the dependent variable, PRT measurements. The regression equation solutions were obtained by the least squares method (18). The present study used regression analysis to investigate linear relationships between PRT measurements, temperature and animal age.

Results and discussion

In this study, the mean amount of tear production for all Holstein cows ($n = 50$) was 32.90 ± 0.73 mm/15 sec. It was 34.47 ± 1.28 mm/15 sec. for group 1 ($n = 23$) and 31.55 ± 0.73 mm/15 sec. for group 2 ($n = 27$). The difference in the mean amount of tear production between group 1 and group 2 was statistically significant ($P < 0.05$).

Although the amount of tear production tended to increase with the increase in the number of births, this tendency was not statistically significant ($P > 0.05$). Average values according to the number of births are given in Table 1. The correlation between the number of births in group 1 and group 2 is shown in Figure 2.

Rising temperature had a negative (-0.290 ± 18) and statistically significant effect ($P = 0.015$) on PRT

Tab. 1. Mean PRT values changing with the number of births

Number of births	Number of animals	Mean PRT values
1	14	31.35 ± 1.09
2	11	32.63 ± 1.42
3	12	33.83 ± 1.77
4	9	34.11 ± 2.18
5	4	32.00 ± 2.08

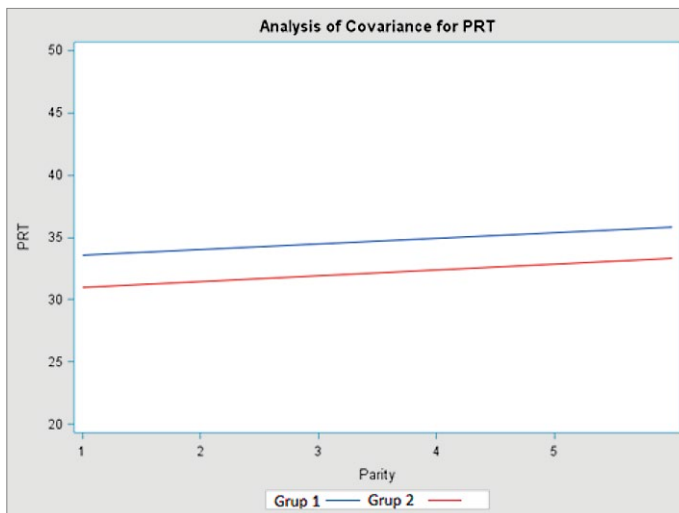


Fig. 2. Correlation between group 1 and group 2 according to the number of births

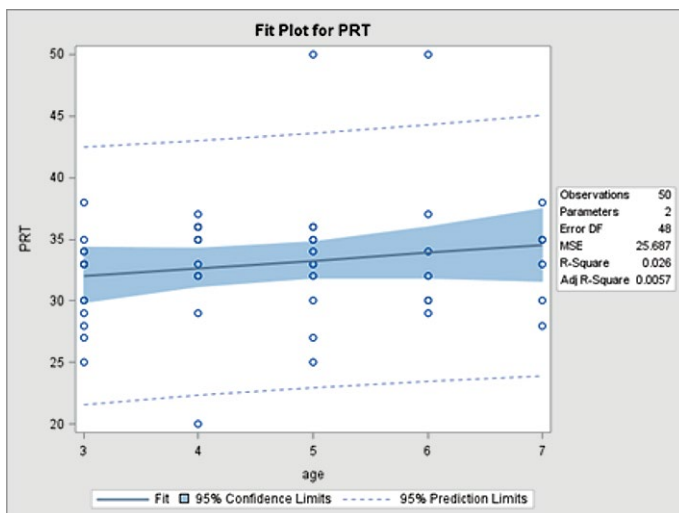


Fig. 3. Linear increase in PRT values by age; regression line and 95% confidence band are given

scores. At high air temperatures, this could be read as a decrease in the PRT score at high temperatures. For example, according to our research, when the temperature climbs from 12°C to 20°C, a 0.29 unit drop in the PRT score can be expected. The ages of the cows and PRT scores showed a slight, but positive correlation (Fig. 3). The effect of linear increase in age, on the other hand, was statistically negligible ($P < 0.05$).

PRT is a diagnostic test developed to replace the Schirmer test to diagnose keratoconjunctivitis sicca (KCS) and tear film abnormalities in domestic animals (7, 14, 37). The results of this study provided information about the effect of seasonal temperature, the number of births and age on the amount of normal tear secretion, as well as the normal value ranges for PRT in Holstein cows.

Many studies have been conducted to determine the mean PRT values in various animal species and breeds (2, 20, 26, 34). The mean STT values in cows were determined to be 24.18 ± 6.5 mm/30 sec. by Whitley R. et al. (36) and 34.15 ± 20.47 mm/min by Wieser B. et al.

(37). Tofflemire et al. (33), on the other hand, reported this value to be 20.4 mm/min in healthy calves, and Suyama et al. (32) found it to be 18.9 ± 2.9 mm/min in Japanese black calves. However, no study has yet provided the mean PRT values for Holstein cows. In this study, the mean amount of tears for Holstein cows in PRT tests was found to be 32.90 ± 0.73 mm/15 seconds (25-38 mm/15 seconds). It is thought that these results may contribute to further studies. It has been reported that the sex of the animal is one of the factors predisposing to keratoconjunctivitis sicca, as this condition is seen more frequently in females than it is in males (6, 27, 37). It has also been shown that there is no significant difference between the right eye and the left eye in terms of the amount of tear secretion (7, 23). Prolactin, a milk secretion hormone, has been found to be an endogenous tear suppressor (9, 20, 25). We could not find a study that looked at the association between prolactin levels and the amount of tears secreted by cows as the number of births increased. It was therefore impossible for us to make a comparison. Although there was a significant increase in the amount of tears as the number of births increased, this tendency was not statistically significant. The amount of tear production did not appear to be affected by the number of births.

Beech et al. (6) reported that the mean STT values for horses were 21 ± 6 mm/min in summer and 26 ± 6 mm/min in winter. This result shows that STT values measured in winter are higher than they are in summer. In another study on sheep, Dedousi et al. (10) found that seasonal increase in air temperature increased the amount of tear production. In this study, when the effect of the seasonal temperature of the environment on tears was examined by the PRT test, the average amount of tears was found to be 34.47 ± 1.28 mm/15 sec. at +12°C in January in group 1 and 31.55 ± 0.73 mm/15 sec. at +20°C in May in group 2. The difference between the means for these two groups revealed that an increase in temperature caused a decrease in the amount of tears, which was statistically significant ($P < 0.05$). These findings are consistent with those by Beech et al. (6). As a result of the present study, it is now known that seasonal temperature changes affect dry eye in Holstein cows.

It has been reported that the prevalence of KCS in humans (34) and dogs (4) increases with age. In the present study, the increase in PRT values with age was not statistically significant (Fig. 3). However, despite its insignificance, it can be concluded that PRT values rise with age. This difference may be related with cows' young and middle ages, as well as to progressively impaired adaptation to environmental conditions as they aged.

In conclusion, this study provides new data and reference ranges for PRT values in healthy Holstein cows. In addition, it reveals that Holstein cows had a lower

PRT score in spring than in winter, that the amount of tears changed with seasonal temperature, and that the number of births and age did not affect tear secretion in young and middle-aged cows.

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