Study on Vaginal Epithelial Cells in Ongole Grade Cattle Suspected Reach Puberty

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ABSTRACT

Puberty is characterized by the time when cattle first estrus. Estrus in cattle can be seen through the cytology of the vaginal epithel (Vaginal smear). The study aimed to assess the vaginal wall cytology of Ongole Grade cattle predicted in the onset of puberty. Ongole Grade cattles were reared by a group of farmers which located in six sub-district: Mirit, Lembu Purwo, Ambal, Puring, Petanahan, and Klirong in Kebumen district, Central Java Province. The estimation of puberty was using 3 nonlinear mathematical models consisted of Gompertz (6.3 months, n = 4), Bertalanffy (7.2 months, n = 4), and Logistics (10.9 months, n = 7). Vaginal smear was done by smear wetted cotton bud using aquadest on Ongole Grade cattle vaginal wall, then it was applied to glass object. The glass objects were immersed in alcohol (70%) for 5 to 7 minutes, then it were immersed in liquid giemsa stain (5%) for 45 minutes. The glass objects were dried, then it were observed using a microscope with magnification 40 times. The vaginal smear samples were taken 8 times, and done every 3 days. The results showed that there were only parabasal and intermediates cell in all ages of cattle observed. This indicates that all the animals observed have not entered the age of puberty.

Keywords: Kebumen, Ongole, Puberty, Smear, Vagina

INTRODUCTION

The success of Zebu cattle matting is closely related to estrus detection ability (Mingoas and Ngayam, 2009). The reproductive efficiency of cattle will rely heavily on the ability of estrus detection, thus affecting the calving interval, and more broadly on production costs. Estrus detection is usually done visually, but their use and efficiency are controversial (Fahey *et al*., 2002). In addition, silent heat in zebu cows is a supplementary difficulty to visual estrus detection, and other techniques such as electronic mounting detection and hormones profile monitoring appear to be very expensive for low income livestock production systems. Differences in the appearance of changes in sexual behavior in each different species result in observing changes in sexual behavior is not enough to determine the right time to mate. Other methods of observing reproductive biology include studying the image of vaginal epithelial (cytologic) cellular changes (Möhle *et al*., 2002).

The observation of the morphology of vaginal epithelial cells is a simple method that practitioners can use to characterize the cycle phase of estrus in livestock and to evaluate various reproductive tract diseases (Dugweker *et al*., 1978; Bishnoi *et al*., 1982). Fluctuations in hormones will affect the image of vaginal epithelial cells. In the luteal phase (the influence of the hormone progesterone), there is a parabasal cell, while entering the estrous phase (the influence of the hormone estrogen) epithelial cells transforms into superficial cells and the kornification that signifies the animal in peak estrus state (Boume 1990). The study aimed to assess the vaginal wall cytology of Ongole Grade cattle predicted in the onset of puberty.

MATERIALS AND METHODS

Data Collection. The estimation of puberty was using 3 nonlinear mathematical models consisted of Gompertz, Bertalanffy, and Gompertz as explained in Table 1 (Gbangboche *et al*., 2011). The data used in this study were body weight of 768 female PO cattle, from the age of birth (< 1 month) to mature (66 months). These cattle are from six sub-districts Mirit, Lembu Purwo, Ambal, Klirong, Petanahan, and Puring. The cattle weight data are collected by PO Cattle Breeder Association (ASPOKEB) from the year 2013 to 2015. The data used consist of body weight and the age of the cattle. Cattle age data was obtained from recordings in each breeder group, which was then calculated to determine the age of each cattle. Body weight data is obtained from recording cattle weighing from the year 2013 to 2015. The weighing of body weight was done using a cattle scale with a 1000 kg capacity and an error margin of 1kg. The type of data used are cross sectional data, in which data collection were performed by measuring the weight of individual cattle in a certain age group, followed by the measurement of other individual samples from within the same population (Fitzhugh, 1976).

Table 1. The equation of three mathematical models

Model	Yt	M	Weight of inflection	Inflection time	Prediction age at puberty (month)
Bertalanfy	$A(1-Be^{-kt})^3$	3	A(8/27)	ln 3B/k	7.2
Logistic	$A(1+Be^{-kt})^{-1}$	-1	A(0,5)	ln B/k	10.9
Gompertz	$Ae(-Be^{-kt})$	$M\to\infty$	$A(e^{-1})$	ln B/k	6.3

Yt = Body weight on t of age, A = Mature body weight (Asimtot), B = The proportion of mature weight which will reached after birth weight formed by Y0 and early t (the value of integral constants), e = Basic of logarithm (2,718282), k = the animal growth rate reach on mature body weight, M = Parameter which obtained the point of inflection in a curve

Determining the onset of puberty. Determining the age and weight at puberty was performed by discovering the inflection point in the growth curve. The inflection point is a maximum point of growth in body weight. At that point, there is a shift change showing the acceleration of the growth becomes to slow down in growth. At that point, the animals reach their puberty (Brody, 1945). Inflection point was performed by using equations of a mathematical model as presented in Table 1.

Vaginal smear. Vaginal smear test was used to determine the best mathematical model for predicting age puberty. Vaginal smear test was conducted by rubbing a cotton bud that has been moistened with aquadest on the walls of the vagina, which then smeared onto glass slides. Next, the slides were soaked in 70% alcohol for 5 to 7 minutes, then it was soaked with 5% Giemsa stain for 45 minutes. Glass slides were then rinsed with aquadest and dried. Colored glass slides were observed with an electron microscope with a magnification of 40 times to observe the development of cells in the vaginal wall (Ahmadi, 2006). Vaginal smear was done every 3 days, for 21 days.

RESULTS AND DISCUSSION

A vaginal smear test was performed to determine the vaginal wall histologic changes in PO heifers that predicted at puberty, the vaginal wall epithelial cell changes affected by the estrogen hormone. The determination of age expected to be puberty was determined based on predicted puberty age using 3 nonlinear mathematical models. Estimated age of puberty (Table 1) using Logistic, Bertalanfy, and Gompertz models were 10.9 months (n=7), 7.2 months (n=4), and 6.3 months (n=4) respectively. A smear of PO heifer vaginal wall epithelium can be seen in Figure 1.

Based on Figure 1, the vaginal wall epithelial cells are seen consisting of parabasal (a) and intermediate cells (b). Parabasal cells have larger nuclei than cytoplasm, generally clustered close together. Parabasal cells are commonly found during diestrus and anestrus (Najamudin *et al*., 2010). The intermediate cells are circular and square irregular, the shape and size of these cells vary but have a diameter of two to three times bigger than parabasal cells, and it found almost at all estrous stages, except at the time of estrus (Nalley *et al*., 2011). Johnston *et al*. (2001) adds that when large numbers of superficial cells are found in the histology of vaginal wall epithelial cells it indicates that the cows are in an estrous condition. Based on the results of histologic observations of vaginal wall epithelial cells it was concluded that overall PO heifers observed were still in the follicular phase or follicular phase development, and had not yet entered the puberty or estrous cycles. The pubertal phase has not been achieved because the observed cattle have not reached the weight of puberty. Bearden *et al*. (2004) suggests that age of puberty is influenced by genetic and environmental factors, where the weight at puberty is more influenced by genetic factors. Genetic factors can be seen by comparing species or breeds. The average age of puberty for European dairy cows is 8 to 11 months, 10 to 15 months in European beef cattle, and 17 to 27 months in Zebu cattle. The weight at puberty among cattle depends on the adult's weight of each cows.

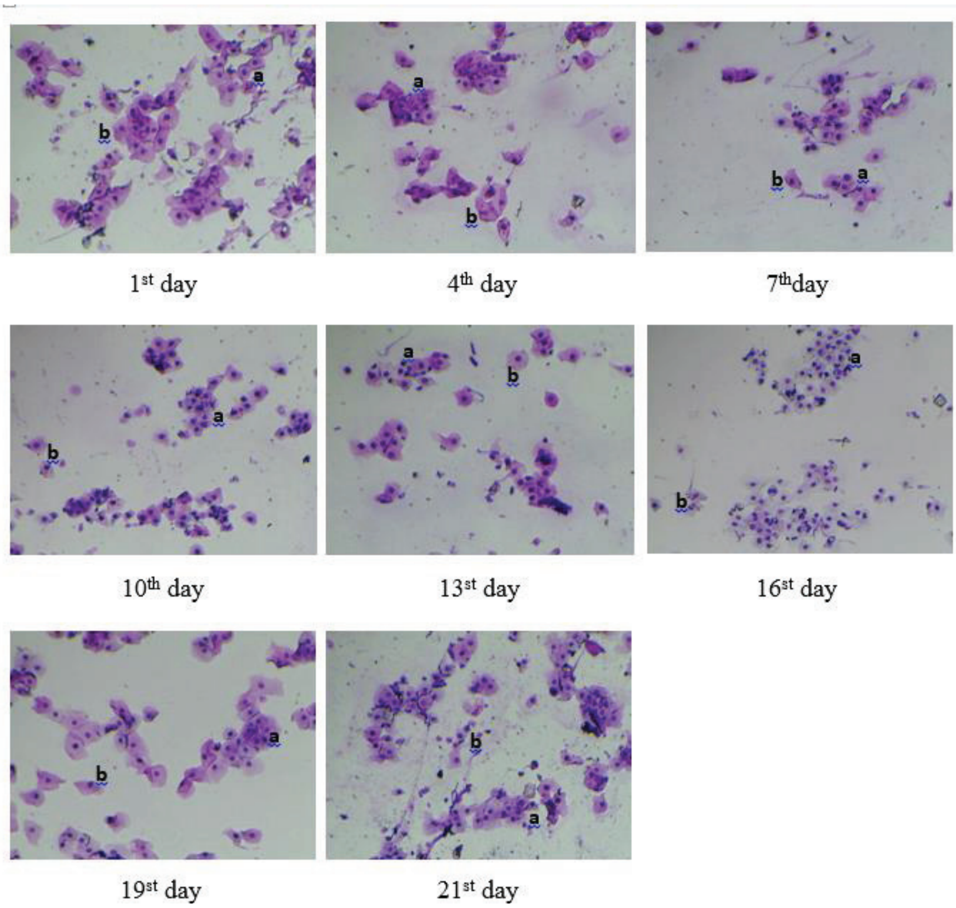

Figure 1. Smear from vaginal wall epithel Brahman heifer (10,28 month)
a. Parabasal cells (nucleated clear, regular round shape and small cytoplasm, b. Intermediate cells (nucleated, irregular shape, and larger cytoplasm.

The first Estrus will occur if the livestock reaches a certain minimum weight rather than reaching a certain age (Rudolf, 2010). Puberty in cows are influenced by genetics (Nogueira, 2004), feeding and weaning age (Guggeri *et al*., 2014), puberty is also affected by maintenance and climate management (Chebel *et al*., 2007). Some environmental factors that affect the age of puberty are nutritional intake, environmental temperature, season, health management and sanitation management. Environmental factors that have a great impact on the age and weight of Zebu cattle puberty are the nutrients and climate in which the cows are kept. Guggeri *et al*. (2014) suggests that limited feeding will result in delayed puberty, whereas supplementation in feeds increases growth and accelerates puberty. Zarate-Martinez *et al*. (2013) adds that the management of livestock raising that affects the quantity, quality, and continuity of feed supplies, especially on before and after weaning will result in delays in the growth of livestock.

CONCLUSIONS

The results showed that there were only parabasal and intermediates cell in all ages of cattle observed. All observed cattle are still in the follicular phase. This indicates that all the animals observed have not entered the age of puberty.

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