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Study on vaginal epithelial cells in Brahman cattle suspected reach puberty

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Abstract Puberty can be characterized by the time when the cattle first estrus. Estrus in cattle can be observed through the cytology of the vaginal epithelial (Vaginal smear). The vaginal wall cytology of Brahman cattle predicted at the onset of puberty was studied. Brahman cattles were developed by Livestock Breeding Center for Excellence and Forage (BPTU-HPT) Sembawa since 2012. The estimation of puberty was using 3 nonlinear mathematical models, there are consisted of Gompertz (10.28 months, n = 4), Bertalanffy (09.96 months, n = 4), and Logistics (15.5 months, n = 7). Vaginal smear was done by smearing wet cotton bud using aquadest on Brahman cattle vaginal wall, then it was smeared to glass object. The object glasses were immersed in alcohol (70%) for 5 to 7 minutes, then it were soaked in liquid Giemsa stain (5%) for 45 minutes. The object glasses were dried, then it were observed using a microscope with a magnification 40 times. The vaginal smear samples were taken 8 times and done every 3 days for each cattles. The results described that there were only parabasal and intermediates cell in all ages of cattle observed. All the animal observation was not entered the age of puberty.

Keywords: Brahman, Estrus, Puberty, Smear, Vagina

Introduction

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). The Study on vaginal ephitelial cells as a useful tool for estrus detection and estrous phase in clinical animals and breeding stations has been described for semo some species and breeds of animals (Leigh *et al*., 2010). The vaginal cytology also can be used clinically to evaluate the hormonal status or to characterize the reproductive stages of animal (Zohara *et*

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al., 2014). Fluctuations in hormones will affect the image of vaginal epithelial cells.

The vaginal epithelium consists of three types of cells which always have cornification, consisting of parabasal cells, intermediate cells and superficial cells (Bologna, 2001). Intermediate and parabasal cells were indicated by vaginal smear in metestrus, diestrus, and proestrus phase. 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 Brahman heifers cattle predicted in the onset of puberty.

Materials and methods

The estimation of puberty was used 3 nonlinear mathematical models consisted of Gompertz, Bertalanffy, and Logistic as explained in Table 1 (Maharani *et al*., 2017). The Mathematical models were interpreted 768 female PO cattles body weight data. The body weight data were collected from the age of birth (< 1 month) to mature (66 months). The Brahman cattle were developed by Livestock Breeding Center for Excellence and Forage (BPTU-HPT) Sembawa since 2012. The cattle weight datas were collected 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 groups, which was then calculated to determine the age of each cattle. 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	09.96
Logistic	$A(1+Be^{-kt})^{-1}$	-1	A(0,5)	ln B/k	15.5
Gompertz	$Ae(-Be^{-kt})$	$M\rightarrow\infty$	$A(e^{-1})$	ln B/k	10.28

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 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 test was done by smearing wet cotton bud that has been moistened with aquadest on the walls of the vagina, which then smeared onto glass object. Next, the object glasses were immersed in alcohol (70%) for 5 to 7 minutes, then it were soaked in liquid Giemsa stain (5%) for 45 minutes. The object glasses were 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 *et al.,* 2006). Vaginal smear was done every 3 days, for each cattles.

Results

The stage of estrus cycle was predicted through morphologic changes in the vaginal ephitelial wall, which had been associated with the level of steroid sex hormones. The vaginal ephitelial cells were classified according to their location in the vaginal mucosa as parabasal, intermediate and superficial cells. The Figure 1 shows the vaginal cell wall from one of the Brahman cows suspected reach puberty (10.28 month). Based on observations, there are 2 types of cells, parabasal cells (a) and intermediete cells (b) and there are no superficial cells. This result was also found at all cattles observed.

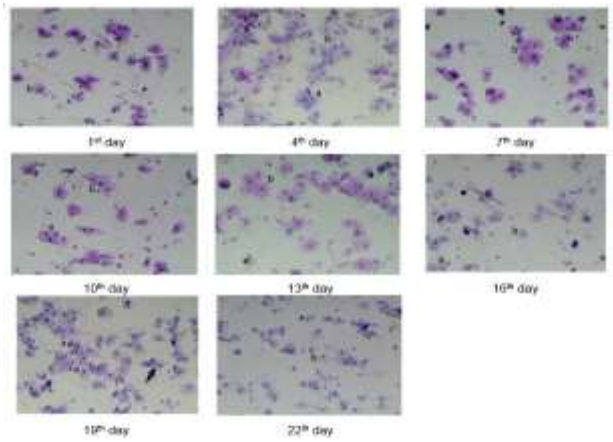

Figure 1. Smear from vaginal wall epithel Brahman heifer (10.28 month). a. Parabasal cells (nucleated clear, regular round shape and small cytoplasm, b. Intermediate cells (nucleated, irregular shape, and larger cytoplasm).

Discussion

A vaginal smear test was performed to determine the vaginal epithelial changes in Brahman 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 15.55 month (n = 7), 9.96 months (n = 5), and 10.28 months (n = 5) respectively. Sharma and Sharma (2016) and Antonov *et al.* (2017) Sitaresmi (2018) reported that parabasal cells are round, small, oval shape, larger nuclei than cytoplasm, generally clustered close together. Parabasal cells are commonly found during diestrus and anestrus. 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). Sitaresmi (2018) stated that intermediate cells are dominate at meteestrus and proestrus phases. Similar results were obtained when observing Ongole grade cattle suspected reach puberty, there were only parabasal and superficial cells in cattle observed (Amrullah *et al.*, 2017).

Perez *et al*. (2009) stated that superficial parabasal cells were specifically marked to detect the activity of ovarium before puberty. Schuttle (2010) adds that superficial cells in vaginal smear were specifically marked for the condition of estrogen hormone. Superficial cells were found on vaginal smear during estrus phase while estrogen concentration was increased (Indira *et al.*, 2014). Johnston *et al*. (2001) stated 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 Brahman female heifers observed were still in the follicular phase or follicular phase development, and had not yet entered the puberty or estrous cycles.

Estrus cycle phase was affected by hormonal change that occured during ovulation with the increase of the luteinizing hormone on bloods. Acosta *et al.* (2003) stated there is significant correlation between estradiol plasma concentration and luteinizing hormone which increased the vascularization within preovulation cycle on cattle. The gonadal hormones (progesterone and estrogen) play important roles in the regulation of the sexual cycle and maintenance of gestation. Estrogen causes the histological changes in vaginal epithelium. Prior to puberty and during anestrous in adults, the vaginal epithelium is only a few cell layers thick, with estrogen stimulation, the vaginal mucosa becomes a stratified squamous epithelium composed of many layers of cells.

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