Comparison of Cow's Breed on the Morphology of Ovaries Collected from Abattoir

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ABSTRACT *In vitro* production (IVP) of embryos is one of the popular animal reproductive biotechnologies to enhance the genetic improvement of livestock. However, insufficiency of good quality oocytes has become one of the major constraints in producing embryos from IVP. Thus, a preliminary evaluation of ovaries could provide information for further experiment regarding IVP of cattle embryos. The objective of the present study was to evaluate the morphology of ovaries from different breed of cattle, including surface follicles and follicular health status. Thirty two (32) ovaries from Kedah-Kelantan breed and 32 ovaries from crossbred cows were collected from an abattoir in Taiping, Perak. Follicles visible on the surface of each ovary were counted and the weight, length and width of the ovaries were measured. The ovaries were then fixed and processed for microscopic evaluation, where the number of healthy and atretic follicles were counted. The mean ovarian weight, length, width and number of surface visible ovarian follicles of crossbred cows were found significantly higher (7.81 ± 0.61 g, 3.30 ± 0.09 cm, 2.31 ± 0.07 cm and 29.66 ± 2.32, respectively) compared to Kedah-Kelantan cows (3.22 ± 0.33 g, 2.60 ± 0.08 cm, 1.80 ± 0.07 cm and 20.97 ± 2.04, respectively). The mean percentage of healthy follicles in the ovaries of Kedah-Kelantan and crossbred did not differ significantly (41.12 ± 5.24 and 48.61 ± 10.15, respectively). In conclusion, breed of cows had statistically significant influence on the surface follicles, but not significant in follicular health status. Further studies of breed factor on oocytes are required for successful IVP of cattle embryos.

KEYWORDS: Breed of cow; Crossbred cow; Follicles; Kedah-Kelantan; Ovarian Morphology
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INTRODUCTION

In Malaysia, livestock industry is one of the important industries in supplying the source of protein for the Malaysian population. Livestock industry consists of ruminant and non-ruminant sectors in which non-ruminant sector, particularly poultry, has reached 100% self-sufficiency, but the ruminant sector is still far behind with below 30% sufficiency level (DVS, 2019). The low production in ruminant sector can be related to the animals having slow genetic improvement and low rate of reproduction. Therefore, the utilization of animal reproduction technologies would be one of the alternatives required to increase the ruminant production.

Until today, various technologies on animal reproduction had been developed and IVP of embryos has been the current technique for embryo production with highly effective results. Freitas & Melo (2010) stated that IVP of animal embryos involve four main steps; the collection of oocytes from follicles, the *in vitro* maturation (IVM) of oocytes, the *in vitro* fertilization (IVF) of the matured oocytes, and the *in vitro* culture (IVC) of the resulting embryos. According to Ayman *et al.* (2016), only 30% to 40% of oocytes achieved blastocysts phase through *in vitro* process which is quite low. Thus, preliminary studies, including the selection of ovaries, oocytes collection, and quantity and quality of ovarian follicles and oocytes harvested for IVM are important to be carried out to improve the IVP of embryos (Kouamo and Kharche, 2014, Kouamo *et al.*, 2014; Hoque *et al.*, 2011).

Cow's breed is one of the important factors to look into during the ovarian selection for IVP. Several studies had reported on the influence of breed on the ovarian follicles and oocytes (Kouamo

et al., 2014; Silva-Santos *et al.*, 2011; Fihri *et al.*, 2004). Hence, the aim of this study is to evaluate the morphology of ovaries, including surface follicles and follicular health status from different breed of cattle.

METHODOLOGY

Collection and macroscopic evaluation of ovaries

Ovarian samples were collected from 32 cows of different breeds [Kedah-Kelantan (16 heads) and crossbred (16 heads)] at a local abattoir in Taiping, Perak. The age of the cows were between 3 to 5 years old with a body condition score (BCS) between 3 to 4 (5-point scale). After slaughtering, both ovaries were collected from each cow and placed in separate labeled vials containing physiological normal saline solution. All ovaries collected were trimmed and cleared off the attached tissues. The collected ovaries then underwent macroscopic evaluation, where each ovary was weighed using electronic weighing balance and measured for length and width using vernier calliper. The number of visible follicles on the ovarian surface was then counted and recorded.

Microscopic evaluation of ovaries

Microscopic evaluation was conducted in the Regional Veterinary Laboratory of Bukit Tengah, Penang and Anatomy and Physiology Laboratory, Faculty of Sustainable Agriculture, Universiti Malaysia Sabah. The ovarian samples were fixed in 10% buffered neutral formalin (Histoline, Puchong, Selangor) for at least 24 hours. Each ovary was divided into eight strips and one strip was chosen randomly for microscopic evaluation of number and healthy status of follicles, as described previously by Modina *et al.* (2013). The chosen strip was dehydrated in isopropyl alcohol, cleared with xylene, embedded in paraffin wax, and serially sectioned at 5 μm thickness. Every 40th section was stained with Hematoxylin and Eosin. For this microscopic evaluation, only 18 ovaries of Kedah-Kelantan and 12 ovaries of crossbred cows from the collected ovaries were selected as the representative ovaries for the respective groups as well as because they displayed complete ovarian cross-section on the prepared staining slides.

The prepared staining slide were observed and counted under microscope (Nikon Eclipse E200) for the number of healthy and atretic follicles. Only follicles with visible oocyte nuclei were counted as healthy and atretic follicles. Follicles were considered healthy if they had organized granulosa cell layers, intact basal membrane, and intact oocyte and nucleus (Ireland *et al.*, 2008; Lussier *et al.*, 1987; Modina *et al.*, 2013; Yang and Rajamahendran, 2000). The follicles were categorized as primordial (one layer of flattened granulosa cells surrounding the oocyte), transitory (one layer of flattened-cuboidal granulosa cells surrounding the oocyte), primary (one to one and a half layers cuboidal granulosa cells surrounding the oocyte), secondary (two or more layers of cuboidal granulosa cells surrounding the oocyte) and antral (six or more layers of cuboidal granulosa cells with a fully formed theca interna and an antral cavity) (Ireland *et al.*, 2008). The total number of follicles in the ovary was estimated by multiplying the counted number of follicles with 320 (40 x 8), a correction factor that takes into account the counting follicles in every 40th section in one of the eight strips (Modina *et al.*, 2013). The percentage of healthy follicles in each follicular category were calculated.

Statistical Analysis

The results in this study were stated as mean ± SE. SAS (1998, Version 6.12) software was used to perform the statistical analysis. The mean value of the two groups were statistically assessed using *t*-tests and a probability of $P<0.05$ considered statistically significant. Pearson's correlation coefficient (r) were used for correlation analysis.

RESULT AND DISCUSSION

A total of 1620 follicles were counted on the surface of 64 ovaries collected from the abattoir. The average weight, length, width and number of visible follicles between KK breed and crossbred cows are shown in Table 1.

Table 1. Ovarian weight, length, width and number of visible follicles of crossbred and Kedah-Kelantan breed cows (Mean ± SE)

Parameter	Crossbred (n=32)	Kedah-Kelantan (KK) (n=32)
Ovary weight (g)	7.81 ± 0.61[a]	3.22 ± 0.33[b]
Ovary length (cm)	3.30 ± 0.09[a]	2.60 ± 0.08[b]
Ovary width (cm)	2.31 ± 0.07[a]	1.80 ± 0.07[b]
Number of visible follicles	29.66 ± 2.32[a]	20.97 ± 2.04[b]

[ab] means with different superscripts in the same row differ significantly (P<0.05).
n = number of ovaries.

From Table 1, crossbred cows have significantly (P<0.05) heavier, as determined by their weight, longer and wider ovaries, compared to the KK breed cows. The mean weight (3.22 ± 0.33 g) of KK ovaries in this study was close to the previous study of purebred Uganda Ankole zebu (4.6 ± 2.3 g) by Natumanya *et al.* (2008). The close findings of ovary weight between the KK and the Ankole zebu cows might be because of both breeds are from the pure *Bos indicus* group. The ovarian weight from crossbred cows (7.81 ± 0.61 g) in this study was similar with the finding of Ireland *et al.* (2008) where crossbred cows with high (≥25 follicles) antral follicle count (AFC) had mean ovarian weight of 7.11 ± 0.41 g. In this study, the mean ovarian length and width of KK cows recorded was comparable with the finding of Kouamo *et al.* (2014) in which Gudali breed cows had ovarian length and width of 2.77 ± 0.05 cm and 1.90 ± 0.03 cm, respectively, which were smaller than that of the crossbred found in this study. Thus, from the findings, we could consider that ovaries from the crossbred cows was heavier, longer and wider than that of the purebred *Bos indicus*. Based on Table 2, there was a positive correlation (P<0.05) between the ovary weight, length and width. Similar results could be expected when relating the results of ovary weight of the purebred *Bos indicus* by Natumanya *et al.* (2008) with ovary length and width by Kouamo *et al.* (2014), where longer and wider ovary are expected to be heavier and vice versa.

The differences in ovarian weight, length and width of the ovaries between breeds can be considered as breed effect and may be related to the live body weight and height of the cattle. Ariff *et al.* (1993) reported that the crossbred females had significantly heavier live body weight than the pure KK females. The correlation between ovary size and cattle size was reported by Eborn *et al.* (2013) as they stated that larger MARC II heifers had larger ovaries, where positive correlations ($P \leq 0.01$) were found between hip height and length of ovaries and between body weight and height of ovaries.

Ovaries of crossbred cow had significantly (P<0.05) higher number of visible follicles than ovaries of KK cows (Table 1). The similar trend was also reported by Peralta-Torres *et al.* (2016) where they found the number of 4.1-8mm follicles were significantly higher in crossbred heifers than pure zebu heifers. Carvalho *et al.* (2008) also reported that the crossbred had higher number of follicles than that of pure *Bos indicus* cows. Another finding by Fihri *et al.* (2005) reported that the Oulmes-Zaers (pure *Bos indicus*) had lower (P<0.05) mean number of follicles (18.96 ± 6.52) compared to crossbred cows (24.71± 8.94). The effects of heterosis, the superiority showed of the crossbred to those of its purebred parents, might be the reason for the higher number of follicles found in the crossbred females than in the pure *Bos Indicus* females. However, there were no available reports regarding the connection of the higher number of follicles with heterosis, only that a study by Johari *et al.* (1994)

where they reported the effects of heterosis on reproductive traits such as calving rate and they suggested the KK crossbred have better efficiency in reproduction compared to the pure KK females.

Table 2. Pearson's correlation coefficients for the number of visible follicles and the ovary weight, length and width of cow ovaries

	Ovary weight	Ovary length	Ovary width	Number of visible follicles
Ovary weight	1	0.72***	0.93***	0.59**
Ovary length		1	0.62**	0.40*
Ovary width			1	0.48**
Number of visible follicles				1

*P<0.05, **P<0.01, ***P<0.001.

There was a positive correlation (P<0.05) between the size of ovaries (weight, length and width) and the number of visible follicles (Table 2). This showed that as the size of the ovaries increases, more possibility for the ovaries to have a higher number of follicles and more chances to have a higher quantity of oocytes. However, according to Eborn *et al*. (2013), although there was a positive correlation between total AFC and ovarian volume, it is not connected with fertility, possibly because of not all the follicles contain healthy oocytes needed for fertilization.

Table 3. Total number of follicles observed microscopically and percentage of healthy follicles of crossbred and Kedah-Kelantan breed cows (Mean ± SE).

Parameters	Breed	
	Crossbred (n=12)	Kedah-Kelantan (KK) (n=18)
Total healthy and atretic follicles	1120.00 ± 241.21^{b}	7253.33 ± 1499.15^{a}
Healthy per total follicles (%)	48.61 ± 10.15^{a}	41.12 ± 5.24^{a}
Healthy primordial per total primordial (%)	45.83 ± 15.02^{a}	43.21 ± 7.08^{a}
Healthy transitory per total transitory (%)	43.75 ± 17.52^{a}	37.92 ± 8.07^{a}
Healthy primary per total primary (%)	66.67 ± 33.33^{a}	21.43 ± 14.87^{a}
Healthy secondary per total secondary (%)	0.00 ± 0.00^{a}	30.00 ± 20.00^{a}
Healthy antral per total antral (%)	66.67 ± 33.33^{a}	66.67 ± 33.33^{a}

^{ab} means with different superscripts in the same row differ significantly (P<0.05)
n = number of ovaries.

Although the ovarian weight, length, width and number of visible follicles on the surface of the ovaries were significantly higher in crossbred than the KK cattle, however, the total number of follicles observed microscopically showed contrast result where KK breed cows have significantly (P<0.05) higher (7253.33 ± 1499.15) total healthy and atretic follicles compared to crossbred cows (1120.00 ± 241.21) (Table 3). The results of the microscopic evaluation of ovaries were in contradiction with the results obtained by counting the number of surface visible follicles since there might be a possibility that not all of the follicles appear on the ovarian surface. The significant difference in the total number of healthy and atretic follicles but similar in the percentage of healthy follicles found in both Kedah-Kelantan and crossbred cows groups indicate that each follicle of ovaries did not necessarily have adequate conditions and accommodates healthy oocytes needed for fertilization, where some will undergo atresia.

AFC was reported as a reliable marker in predicting the number of healthy ovarian follicles in cattle (Ireland *et al*., 2008). However, in this present study, the number of visible surface follicles did not predict the healthy status of follicles in the ovary since there was an absence of correlation. There

was no significant correlation was found between the number of visible follicles and the total percentage of healthy follicles nor between the percentage of antral follicles and total percentage of healthy follicles. Previous findings observed by Torres-Rovira *et al.* (2013) in ewe lambs also reported no correlation between number of antral follicles and total number of healthy follicles. As for now, we could not explain on the absence of correlation between the number of visible follicles on the ovarian surface and the healthy follicles microscopically counted.

Table 3 also shows the total number of healthy follicles as a percentage of total follicles did not differ between the two breed groups. In addition, there were no significant differences in the percentage of healthy follicles between the two breed groups of primordial, transitory, primary, secondary and antral follicles. The result of healthy follicles was similar to the finding reported by Silva-Santos *et al.* (2011) where there was no significant difference of the overall population of healthy preantral follicles (primordial, primary, secondary) between *Bos indicus* and *Bos taurus* females. The percentage of healthy follicles in all follicular stages analyzed was similar between Kedah-Kelantan and crossbred cows groups in this current study showed that the two groups had a comparable ovarian reserve that appoints the ovarian capacity in providing oocytes that are capable of fertilization and producing embryos.

CONCLUSION

The findings of this study could provide early information for further experiment regarding IVP of cattle embryos. Based on the findings, we inferred that the number of visible surface follicles does not depend on the percentage of healthy ovarian follicles. The weight, length, width and number of visible ovarian follicles were significantly higher in the crossbred cows than that of the purebred cows, but the percentage of healthy follicles were similar in both groups. Therefore, further research studies are required to evaluate whether breed of cows influence the quality of oocytes, quantity of oocytes reaching blastocyst stage and fertilization rate for successful IVP in Malaysia.

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