



Qualitative Evaluation of Slaughterhouse Bovine Ovaries by Histological Study

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ABSTRACT

The aim of this study was to evaluate the morphological and histomorphological attributes of different stages of follicular population of bovine ovaries. Total twelve ovaries were collected from Brahman cows and classified as ovary with corpus luteum (CL) and ovary without CL group. Among the ovaries, 75% having no CL and 25% having CL. The mean weight of ovaries with CL and ovaries without CL were 8.47 ± 0.38 and 8.19 ± 0.20 g, respectively. The number of visible follicles on the surface of the ovaries without CL (26.04 ± 0.19) were significantly ($P < 0.01$) higher than that of ovaries with CL (23.00 ± 0.08). Histological study further reveals that total number of follicles (198.10 ± 0.46) and number of primordial (93.50 ± 0.62), primary (69.40 ± 0.36) follicles significantly ($P < 0.01$) higher in ovaries without CL than those of ovaries with CL (170.14 ± 0.56 and 76.53 ± 0.40 , 61.37 ± 0.62), respectively. Besides this, ovaries without CL contains greater number of normal follicles (8.37 ± 0.09) that having well-defined granulosa cell integrity and lower number of abnormal follicles (26.50 ± 0.61) compared ovaries with CL (4.07 ± 0.09 ; 31.53 ± 0.38). This study inferred that presence of CL may have negative impact on ovarian follicular development. Therefore, it is possible to obtain a higher number of normal quality follicles from the ovary devoid of CL for bovine biotechnological research.

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Introduction

Livestock productivity greatly hampered by two distinct reasons of poor genetic makeup and reproductive failure (Nandi *et al.*, 2006). On that point, scientific upliftment of assisted reproductive technologies like embryo transfer, estrus synchronization, oocytes pick up from live animals, cryopreservation of embryos, in vitro maturation, fertilization, embryo development and cloning bring it possible to overcome these limitations (Verma *et al.*, 2012). Although reproductive technologies are profoundly playing emphatic roles in the production, reproduction management of animals but the basic knowledge of reproductive physiology places elemental part for appropriate and successful application of these techniques. It was also reported by Siddiqui *et al.* (2005) that normal anatomical structure of genital organ of the animals is the vital consideration of its reproductive performance.

Ovary is the principal female reproductive organ which has pivotal importance in fertility and hence reproductive biology (Devender *et al.*, 2013). Follicle is the elemental functional unit in the ovary encompassing the developing oocyte accompanied by one or several

layers of granulosa cells (Tu *et al.*, 2014). Multiple publications have reported that in mammals, a female is born with the pool of primordial follicles and before reproductive senescence, its development has been continued and finally follicles are fated to ovulate and formed CL or degenerate through atresia (Skinner, 2005). Evolvement of corpus luteum is allied to viviparity in mammals which is responsible for secretion of progesterone that is indispensable for growth and maintenance of pregnancy (Juengel *et al.*, 1999). On the other hand, follicular atresia has been depicted as a customary phenomenon in the ovary (Rajakoski, 1960). The aptitude of follicles to synthesize estrogen is a reasonable indication whether that follicle is atretic or not (Ireland, 1979).

Ovarian features of presence or absence of corpus luteum, size and weight of the ovary greatly influenced ovarian follicular population. It is essential to have clear understanding about the two paramount reproductive parameters such as biometry and follicular population on ovaries which ultimately allude reproductive potentiality of animal (Jaji, 2012). The best way of discerning actual populations of follicles, their

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classification and condition is counting of follicles on the ovarian surface and histological study of ovary and slaughterhouse material is the best source regarding this purpose of study (Rind *et al.*, 1999). Histological study of the ovary has given the cellular qualitative status of the follicles. The follicle contains the cumulous-oocyte-complexes (COCs). The quality of the COCs is one of the important factors for in vitro maturation of the oocyte. In Malaysia, as yet, no study has been reported on evaluation of slaughterhouse ovaries, follicular population or status for assessment of the bovine reproductive potentiality which is the fundamental need for successful application of reproductive technologies. So, the present study intended throws some lights on qualitative and quantitative aspects of slaughterhouse bovine ovaries by histological study as it forms the basis for investigation of reproductive efficiency and flourishing productivity of cattle in Malaysia.

Materials and Methods

The experiment was conducted at the Anatomy and Physiology Laboratory, Faculty of Sustainable Agriculture, University Malaysia Sabah (UMS) of Sandakan campus from August 2016 till October 2016.

Collection and processing of ovaries

The ovaries were collected from the Brahman cows which have been culled and slaughtered at local slaughterhouse. The excessive tissues had been trimmed out from the ovaries and placed in the falcon tube contained 35% of formaldehyde solution at room temperature and transported to the laboratory immediately.

Measurement of weight of ovary and follicle counting

The ovaries were then transferred in the sterilized petridish containing same solution (35% formaldehyde) at room temperature and categorized on the basis of the presence or absence of corpus luteum and recorded. The weight of the ovary was measured individually by using electronic balance and recorded in a tabular form. In each and every ovary, the numbers of follicles on the surface of the ovaries were counted carefully by that visible on naked eyes.

Histological sample preparation

The ovaries were cut into small pieces with surgical blades and forceps then fixed into the container (falcon tube) containing chemical mixture that composed of 37-40% formaldehyde and glacial acetic acid for 24 hours or long until further treatment. Dehydration of tissue sample was carried out by upgrading the alcohols to absolute alcohol (50%, 70%, 80%, 95%, 100%) and cleaned with the clearing agent (xylene or chloroform). Thereafter, ovarian parts were transferred into a tube

containing 50% paraffin and 50% xylene and placed the whole in the oven set at temperature about 50-60°C for 2 hours and embedded with melted pure paraffin and kept for hardening.

Section cutting and staining

The blocks were cut by using sliding microtome at 6 µm thick and placed in floatation water bath (55-56 °C) soaked with gelatin at every 10th section picked upon glass slide and dried in air before staining. The section on the slides was dehydrated with graded ethanol, stained with haematoxyline and cleaned in xylene and finally mounted with DPX and covered with the slip.

Microscopy and histoplanimetry

The prepared samples examined under microscope for identification of granular cells and other follicles. The follicles were counted by using microscope at the magnification of 40x. The different stages of the follicles also been taken into count to make some correlated results (Akers and Denbow, 2008, Rodger and Irving, 2010, and Cushman *et al.*, 2009). Besides that, based on the cell integrity of the ovaries number of normal and abnormal follicles were counted using microscope at a magnification of 100x with oil drop.

Statistical analysis

The data was compiled, tabulated and analyzed based on the objectives of the study. The data was analyzed with the help of Statistical Analysis System (SAS) software Version 9.1.3 (SAS, 2009). All data were subjected to one-way ANOVA and statistical significance of difference between the groups was evaluated by Duncan's multiple range test (DMRT). Pearson's correlation was performed to compare among different types of follicular correlation with regards to presence or absence of CL in ovary.

Results

Qualitative evaluation of bovine slaughterhouse ovaries

The bovine ovaries were collected from the slaughterhouse then observed and categorized as ovary with CL and ovary without CL group. Total twelve ovaries were collected, where CL was found in three ovaries and remaining nine ovaries having no CL. So, it was implicated that 75% of ovaries were found without CL and 25% with CL (Table 1).

Weight of ovaries and number of visible follicles on surface of ovary

The mean weight of different categories (ovary with CL and ovary without CL) of ovaries were tabulated and shown in Table 1. The result showed that the weight of the ovaries with CL and without CL were 8.47±0.38g and

8.19±0.20g, respectively though the results not differ statistically. Total number of follicles visible on the surface of ovaries in relation to presence or absence of CL was counted as shown in Table 1. The results of the study revealed that ovaries with CL, pertained lower number of visible follicles (23.00±0.08) than the ovaries without CL (26.04±0.19). Number of visible follicles significantly (P<0.01) differ in ovaries with CL and ovaries without CL group and it was found that number of visible follicles notably affected by the presence or absence of CL.

Table 1. Mean (± SE) number of surface follicles, ovary weight and percentage of type of ovaries retrieved from slaughterhouse

Ovary group	Retrieved (%)	Wt. of ovary (g)	Visible follicles
Ovary with CL	25.0 (3)	8.47±0.38(3)	23.00±0.08 ^b (3)
Ovary without CL	75.0 (9)	8.19±0.20 (9)	26.04±0.19 ^a (9)

CL, corpus luteum; SE, standard error; Values in the parentheses indicate the number of observations. Values in the same column with different small letters superscripts (a, b) differ significantly (P < 0.01).

Histological evaluation of ovarian follicles

Histologically different stages of follicles in relation to the presence or absence of CL was counted and tabulated in Table 2. Total number of follicles was compared with respect to ovary with CL and ovary without CL, there found total number of follicles was lower (p<0.01, Table 2) in ovaries with CL than ovaries without CL with a mean of 170.14±0.56 and 198.10±0.46 respectively. When considering each type of

follicles in both types of ovaries (ovaries with CL and ovaries without CL), ovaries without CL had a significantly higher number of primordial (93.50±0.62, P<0.01) and primary (69.40±0.36, P<0.01) follicles than those of ovaries with CL (76.53±0.40 and 61.37±0.62, P<0.01) respectively. However, the number of secondary follicles did not differ between two groups of ovaries (P> 0.05).

In ovary with CL, there was strong positive correlation persisted between primary follicle and secondary follicles (r = 1, p<0.01) and number of primary and secondary follicles positively correlated with the total number of follicles (p<0.05, Table 3). whereas, in ovary without CL, correlations between the number of primordial or primary follicles, Secondary follicles and total follicular populations were not significant (Table 3).

The integrity of the granulosa cells been considered as the parameter on identifying the abnormality and normality of the follicles. A high integrity or well-defined granulosa cells surrounded the oocyte was recognized as normal follicles while abnormal follicles are vice versa. Total number of normal and abnormal follicles in ovaries with CL and ovaries without CL group were summarized in Table 4. Histological examination revealed that significantly (p<0.01) higher number of normal follicles were found in ovaries without CL (8.37±0.09) compared to ovaries with corpus luteum (4.07±0.09) while, higher number of abnormal follicles were found in ovaries with CL (31.53±0.38) than in ovaries without CL (26.50±0.61).

Table 2: Mean (± SE) number of microscopic counted follicles per ovary

Ovary group	Number of follicles per ovary			
	Primordial	Primary	Secondary	Total
ovary with CL (3)	76.53±0.40 ^b	61.37±0.62 ^b	34.37±0.62	170.14±0.56 ^b
Ovary without CL (3)	93.50±0.62 ^a	69.40±0.36 ^a	35.43±0.69	198.10±0.46 ^a

CL, corpus luteum; Values in the same column with different small letters superscripts (a, b) have differ significantly (P < 0.01). Parenthesis indicates the number of observations.

Table 3: Pearson's correlations among total number of different stages follicles

	Ovary with CL				Ovary without CL			
	Primordial	Primary	Secondary	Total	Primordial	Primary	Secondary	Total
Primordial		-0.97 ^{NS}	-0.97 ^{NS}	-0.97 ^{NS}		-0.99*	-0.95 ^{NS}	0.89 ^{NS}
Primary			1.00**	1.00*			0.94 ^{NS}	-0.90 ^{NS}
Secondary				1.00*				-0.72 ^{NS}

** Significant at P<0.01, * Significant at P<0.05 and NS, Non-significant (P>0.05)

Table 4: Effect of presence or absence of CL on the mean (±SE) number of normal and abnormal follicles in ovaries

Granulosa Cell Layer	Abnormal	Normal
Ovary with CL	31.53±0.38 ^a (3)	4.07±0.09 ^b (3)
Ovary without CL	26.50±0.61 ^b (3)	8.37±0.09 ^a (3)

CL, corpus luteum; Values in the same column with different small letters superscripts (a,b) have differ significantly (P < 0.01). Parenthesis indicates the number of observations.

Discussion

The average frequency of ovary with CL found 25% in this study, suggesting that about 75% of cattle were either non-cycling or less reproductive performer. This condition explicated that in Malaysia most of the cattle culled and slaughtered on economic point of view. Similar trend of results found in case of cattle (Khandoker *et al.*, 2016), buffaloes (Khandoker *et al.*, 2011), goats (Asad *et al.*, 2016; Hoque *et al.*, 2009, Mondal *et al.*, 2008; Islam *et al.*, 2007). Those findings supported that during random sampling, there had been great probability of assembling higher no of ovaries without CL collected from the slaughterhouse.

The mean weight of ovary, in the present study were found numerically higher in ovaries with CL than those from ovaries without CL but no significant ($p>0.05$) differences were found with these two groups of ovaries. In contrast, with the results came from morphometric measurements of ovaries from cattle by Mahzabin *et al.* (2020), goat by Asad *et al.* (2016), buffalo by Khandoker *et al.* (2011) revealed that there was significant difference existed between ovaries with CL and ovaries without CL in relation to weight of the ovary and ovary with CL exerted higher weight than those ovary without CL. This discrepancy might be attributed due to lower number of sample evaluated.

Moreover, in compliance with previous studies (Rexroad and Casida, 1977; Dufour *et al.*, 1972), there had been a relationship between the development of CL and the development of follicles. In the current study, the number of visible follicles on surface of ovary without CL is 13% higher than the ovary with CL. Besides that, the microscopic counted follicles from the histology slides also showed that the total number of follicles counted from the ovaries without CL was significantly ($P<0.05$) higher (14%) than the ovaries with CL. From these observations it was reported that the presence of CL is influencing the number of visible and histologically counted follicles and this statement likened with the findings of Hajarian *et al.* (2015) who reported that in dairy cows, the presence of corpus luteum was believed to affect estradiol concentration, quantity and quality of follicles. As corpus luteum is the chief source of progesterone hormone that inhibits follicular growth is through suppression of LH pulse frequency (Flynn *et al.*, 2000; Forde *et al.*, 2011). Furthermore, ovary without CL, the situation is reversed where the negative effect of progesterone might not be functional and estrogen-progesterone remain in balanced level which allows follicular growth and oocytes maturation thus contained higher number of normal follicles.

Correlation analysis depicted that among different stages of follicles from both types of ovaries, a positive linear relationship existed between primary follicles and secondary follicles. While in ovaries without CL, number of primordial follicles and total number of follicles also positively correlated. Results of the present study combined with those of previous studies (Cushman *et al.*, 1999; Mahzabin *et al.*, 2020) suggest that the greater activation of the of primordial follicles from the growing pool reflects the potential of ovary to generate further stages of follicular development.

Again, when follicles were characterized in normal and abnormal groups on the basis of integrity of granulosa cell, greater number of normal follicles found in ovary without CL than the ovary with CL and the results revealed that the significant ($p<0.01$) difference subsisted between two groups of ovaries (ovary with CL and ovary without CL) in case of normal follicular population. The causes of higher number of normal follicles found in ovary without CL than those of ovary with corpus luteum can be well explained by the hormonal effects in CL. As it is well known that presence of CL in the ovary leads to higher level of progesterone hormone production which negatively affect follicular growth and also responsible for follicular degeneration (Hafez, 1993). Thus, the ovary not bearing CL far away from the negative effect of progesterone hormone escorts higher no of normal follicles. Conversely, when the number of abnormal follicles compared between two groups of ovaries (ovary with CL and ovary without CL) there was significant difference ($p<0.05$) observed and ovary without CL belongs to 15% lower abnormal follicles than ovary with CL. Here, also fits the previous statement given by Hafez (1993) about the effect of progesterone hormone in relation to CL. Current study strongly supports the previous studies done by Islam *et al.* (2007) and Asad *et al.* (2016) in goat ovaries.

Conclusion

From the present study, it is concluded that ovaries without CL contributing not only higher number of follicles but also greater number of normal follicles that having good quality granulosa cell integrity compared to ovaries with CL. Hopefully this finding will provide a baseline information for further studies to improve livestock reproductive efficiency in Malaysia.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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