

THE EFFECT OF BREED AND BODY CONDITION SCORE ON OVARIAN WEIGHT, NUMBER OF FOLLICLES, AND OOCYTE QUALITY IN RETIRED COWS

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ABSTRACT

The aim of this study was to determine whether breed and body condition score (BCS) can influence ovarian weight, follicle number, and oocyte quality. This study used 90 ovaries from 15 Friesian Holstein crossbreed, 15 Ongole crossbreed, and 15 Limousin crossbreed cows obtained from an abattoir. Before the ovaries were taken, the BCS of the cows was determined. The BCS system used in this study is BCS with a scale of 1 to 5. Ovaries were weighed, follicles were measured and grouped by size (<2 mm, 2-6 mm, >6 mm) then counted, oocytes were aspirated from the follicles then observed under a microscope. Oocyte quality was observed and then grouped into grade A, B, C, D based on cumulus oocyte complex (COCs). Data were analyzed using two way ANOVA and chi square test. The results of the analysis showed that breed did not influence ovarian weight, but influenced the number of follicles, and oocyte quality. In Friesian Holstein crossbreed cows, BCS affects ovarian weight, follicle number, and oocyte quality. In Ongole crossbreed cows, BCS had no effect on ovary weight, but did affect follicle number and oocyte quality. In Limousin crossbreed cows, BCS had no effect on ovary weight and follicle number, but did affect oocyte quality.

Key words: body condition, breed, follicles, oocytes evaluation, ovary

ABSTRAK

Tujuan penelitian ini adalah mengetahui pengaruh breed dan body condition score (BCS) terhadap berat ovarium, jumlah folikel, dan kualitas oosit. Penelitian ini menggunakan 90 ovarium dari 15 sapi persilangan Friesian Holstein, 15 sapi persilangan Ongole, dan 15 sapi persilangan Limousin yang diperoleh dari tempat pemotongan hewan. Sebelum ovarium diambil, nilai BCS sapi ditentukan. Sistem BCS yang digunakan dalam penelitian ini adalah BCS dengan skala 1-5. Ovarium ditimbang, folikel diukur dan dikelompokkan berdasarkan ukuran (<2 mm, 2-6 mm, >6 mm) kemudian dihitung, oosit disedot dari folikel kemudian diamati di bawah mikroskop. Kualitas oosit diamati kemudian dikelompokkan menjadi grade A, B, C, D berdasarkan cumulus oocyte complex (COC). Data dianalisis menggunakan ANOVA dua arah dan uji chi square. Hasil analisis menunjukkan bahwa breed tidak memengaruhi berat ovarium, tetapi memengaruhi jumlah folikel dan kualitas oosit. Pada sapi persilangan Friesian Holstein, BCS memengaruhi berat ovarium, jumlah folikel, dan kualitas oosit. Pada sapi persilangan Ongole, BCS tidak memengaruhi berat ovarium, tetapi memengaruhi jumlah folikel dan kualitas oosit. Pada sapi persilangan Limousin, BCS tidak memengaruhi berat ovarium dan jumlah folikel, tetapi memengaruhi kualitas oosit.

Kata kunci: skor kondisi tubuh, breed, folikel, kualitas oosit, ovarium

INTRODUCTION

The slaughter of female cattle is still widely practiced in Indonesia. The slaughter of female cattle occurs for various reasons, including the reason that the price of female cattle tends to be cheaper than male cattle, supported by the principle of slaughtering at the slaughterhouse, namely the more animals are slaughtered, the more their wages will be (Nielsen *et al.* 2019). Thus, more animals are slaughtered even though the females are productive. Meanwhile, the slaughter of female cattle that have been culled often occurs because they are more than eight years old, have diseases, the economy of the breeder, the cattle are no longer productive, and have physical

disabilities. Therefore, the culling of female cattle occurs because the livestock does not meet the technical requirements according to applicable standards so that it is allowed to be slaughtered and does not violate applicable laws.

Given the large number of female cattle slaughtered at the Slaughterhouse, it would be better if their ovarian waste could be utilized. The ovary is the primary reproductive organ that is capable of producing oocytes (Araújo *et al.* 2014). Utilization of ovarian waste can provide oocytes in large quantities without requiring a lot of costs. Through in vitro fertilization (IVF) technology, oocytes from cow ovarian waste can be processed to produce embryos in vitro which can then be transferred to recipient

livestock that have been prepared to produce calves that have characteristics in accordance with mothers that have superior genetic records, such as good reproductive ability and high milk production, but have been slaughtered due to illness or injury (Currin *et al.* 2021).

In addition, the collected oocytes can be used as stock for research and training related to IVF. IVF and related technologies hold great potential for local cattle industries by improving genetic traits, reproductive efficiency, and addressing fertility issues. However, ongoing research and technological advancements are essential to overcome current limitations and fully realize the benefits of these technologies (Ferré *et al.* 2020). IVF technology is a technique of ovum cell penetration by spermatozoa in a culture system and is one of the reproductive techniques that is useful for increasing the efficiency of sperm and ovum utilization (Chatiza *et al.* 2013). Sperm and ovum are brought together in a tube or petri dish with special treatment first so that fertilization can occur (Shari 2022). In oocytes that undergo the maturation process, Transforming Growth Factor (TGF- β) plays a very important role in the meiosis process, this is related to increasing the quality of the oocytes produced and furthermore also greatly affects the fertility rate (He *et al.* 2021). Therefore, before IVF is carried out, it is important to select oocytes in order to obtain oocytes with good quality, namely oocytes that have a cumulus complex.

Ovarian weight and number of follicles are also things that need to be considered because ovaries, follicles, and oocytes are related. The selection of ovarian waste, follicles, and oocytes to be used for IVF will be easier if before collection the factors that influence it are considered (Aguila *et al.* 2020). By understanding these factors, potential ovarian waste can be obtained for use in IVF because it has the potential to have follicles and oocytes with good quality, thus increasing the chances of IVF success. Ovarian weight in cows can be influenced by various factors including breed, age, body condition score (BCS), and pregnancy status (Berry *et al.* 2003). However, there is variability between individuals and breeds so that the correlation between breed and ovarian weight is not always significant.

Related to follicles, each breed of cattle has a different follicle size development period and in these different follicle sizes can be accompanied by differences in oocyte quality. Meanwhile, oocyte quality is also influenced by factors of breed, age, body weight, BCS, season, physiological, nutritional, and pathological status of the female (Dorice *et al.* 2019). Among these factors, there are factors that are quite easy to observe, namely cattle breed and BCS. In Indonesia, there have not been many studies showing the effect of cattle breed and BCS on ovaries, follicles, and oocytes. The purpose of this study was to determine whether there was an effect between cattle breed and ovary weight, number of follicles, and oocyte quality.

MATERIALS AND METHODS

Research design

Research and observation of oocyte and follicle preparations were conducted in the obstetrics laboratory of the Faculty of Veterinary Medicine, Airlangga University, Surabaya. The research was conducted from January to March 2024. The research sample used was the ovaries of culled female cows obtained from the slaughter of culled female cows at the Surya Slaughterhouse on Pegirian Street, Surabaya City. Ovarian samples came from 3 breeds of cattle, namely Limousin crossbreed, Ongole crossbreed, and Friesian Holstein crossbreed. The sample size used was 30 ovaries from 15 Limousin crossbreed cows, 30 ovaries from 15 Ongole crossbreed cows, and 30 ovaries from 15 Friesian Holstein crossbreed cows, so that a total of 90 ovaries were obtained.

Ovarian Removal Procedure

Identify the breed of cattle to be slaughtered, then pay attention to the anatomical areas of the cow's body to determine its BCS. After the female cow is slaughtered, the ovaries are immediately separated from the cow's body to maintain its freshness. The ovaries are cleaned of attached fat and then rinsed using a 0.9% NaCl solution that has been added with the antibiotic penicillin streptomycin (1 mL/L). Furthermore, the ovary sample is placed in a plastic bag containing a 0.9% NaCl solution containing antibiotics. The plastic is stored in a cooler bag during transportation from the slaughterhouse to the laboratory.

Procedure for Determining Ovarian Weight and Number of Follicles

The ovaries were weighed using an analytical balance. The follicle size was determined using a digital vernier caliper and then grouped into 3 categories, namely small (<2 mm), medium (2-6 mm), and large (>6 mm) (Syaiful 2021). After that, the number of each group of follicles was calculated.

Oocyte Collection Procedure

The technique used for oocyte collection from the ovaries is the aspiration technique. The oocyte aspiration procedure is in accordance with the Indonesian national standard (SNI) listed in the Standard Operating Procedure of the Production and Application Section of the Cipelang Livestock Embryo Center. The ovaries are cleaned and washed from ligaments and organs that are still attached with the ovary handling media then placed in a beaker glass with the same media, after which the beaker glass is placed on a heating plate so that the temperature remains stable at 37.5 °C. Oocyte aspiration from the ovaries uses a 5 mL syringe and an 18G needle that has been filled with aspiration media (3 mL of 0.9% NaCl). Follicles with a diameter of 1 mm > 5 mm are punctured in the stroma with a needle, then the oocytes and follicle fluid are aspirated. The aspiration results obtained are collected in a 90 x 100 mm petri dish. Oocyte search is carried

out using a stereo microscope, oocytes that have been classified according to their grade are placed in a 35 x 10 mm petri dish containing aspiration media.

Oocyte Quality Evaluation Procedure

Aspirated oocytes are grouped based on visual assessment of cumulus cell compactness. According to Handarini *et al.* (2014) the morphological quality of oocytes is categorized into 4 grades, namely: Grade A oocytes/quality A (complete): Oocytes that have uniform and compact cumulus surrounded by five or more layers of cumulus cells. Grade B oocytes / quality B (Expanded): Oocytes that have dark cytoplasm with a complete complement of corona radiata but surrounded by no more than five layers of cumulus cells. Grade C oocytes / quality C (Partial): Oocytes characterized by less uniform oocytes, surrounded by less than two layers of cumulus cells, the color of the cytoplasm is more transparent, uneven and looks not compact. Grade D oocytes / quality D (Nude): Oocytes characterized by transparent cytoplasm, even fragmentation in the cytoplasm, cumulus cells surrounding the oocyte look very rare, even some oocytes do not have cumulus cells.

Data Analysis

The collected data were analyzed using the two-way ANOVA method to determine whether or not there was an effect of the cattle breed on ovary weight, number of follicles, and oocyte quality. After the two-way ANOVA test was carried out, if there was an effect, it would be continued with the Duncan test. In addition, the chi square test was used to determine the effect of BCS on ovary weight, number of follicles, and oocyte quality in each cattle breed.

RESULTS AND DISCUSSION

Effect of Cow Breed on Ovarian Weight

The data in Table 1 for the effect of breed on ovary weight were analyzed using the two way ANOVA Test, then it was concluded that the breed of cattle did not affect ovary weight. Although the results of the analysis showed that there was no effect of each breed of cattle on ovary weight, but if seen from the data, it is known that each ovary from the breed of cattle has a varying ovary weight.

The largest average ovarian weight on the left side of the ovary was found in Limousin crossbreed cattle, which was 1.96 g, followed by Friesian Holstein crossbreed cattle at 1.88 g and the smallest was in Ongole crossbreed cattle at 1.64 g. On the right side of the ovary, the largest average weight was found in Friesian Holstein crossbreed cattle, which was 2.06 g,

followed by Limousin crossbreed cattle at 1.97 g and finally Ongole crossbreed cattle at 1.94 g. The results showed that the average weight of the right side of the ovary was greater and was found in Friesian Holstein crossbreed cattle. However, there was no significant difference between the average weight of the ovary based on the breed of cattle or the side of the ovary.

The results of the study showed that the breed of the cow did not affect the weight of the ovary. In other words, it is not the breed that affects the weight of the ovary, but other factors such as body weight and age. Meanwhile, the potential weight of the ovary of each cow is influenced by genetic selection, reproductive phase, age, birth weight, environmental factors, hormones, follicle population, and corpus luteum (CL).

Genetic selection for fertility and reproductive performance can impact ovarian weight in cows. Studies have shown that cows selected for ovulation and multiple births have larger ovaries than cows not selected. The reproductive stage of the cow can also affect ovarian weight (Murasawa *et al.* 2005). Studies have found that ovarian weight increases during the estrous cycle, particularly during the follicular phase. As cows age, their ovarian weight tends to increase due to follicle accumulation and CL development. Birth weight has been shown to be positively correlated with ovarian weight in cows. Cows with higher birth weights tend to have larger ovaries.

Environmental factors such as nutrition and management practices can also affect ovarian weight. Cows fed a diet rich in energy and protein tend to have larger ovaries than cows fed a diet limited in energy and protein. In addition, hormonal influences, such as luteinizing hormone (LH), are known to stimulate the growth and development of follicles in the ovaries, leading to increased ovarian weight (Rabiee *et al.* 1999).

The number of follicles present in the ovary can also affect its weight. Ovaries with more follicles tend to be heavier than ovaries with fewer follicles (Murasawa *et al.* 2005). The CL is a temporary endocrine structure that forms in the ovary after ovulation and plays an important role in embryonic development. The size of the CL can affect the weight of the ovary, especially during the luteal phase of the estrous cycle (Mahzabin *et al.* 2020). In summary, while breed does not significantly affect ovary weight, other factors such as reproductive stage, age, and nutritional status play a crucial role in ovarian development.

The Influence of Cow Breed on the Number of Follicles

Table 2 shows that the average number of follicles varies across cattle breeds. Friesian Holstein crossbreed cattle had the highest number of small follicles (average

Table 1. Mean ($\pm$ standard deviation) of cattle breeds for ovary weight

Types of cattles	Average ovary weight (g)	
	Left	Right
Friesian Holstein crossbreed	1.88 $\pm$ 0.296	2.06 $\pm$ 0.37
Ongole crossbreed	1.64 $\pm$ 0.47	1.94 $\pm$ 0.51
Limousine crossbreed	1.96 $\pm$ 0.383	1.97 $\pm$ 0.36

of 3), followed by Ongole crossbreed cattle, with Limousin crossbreed cattle having the lowest number. Analysis of variance revealed that cattle breed significantly affected ($P<0.05$) the number of follicles, particularly in large and small follicle size categories.

The average number of follicles was highest in Friesian Holstein crossbreed cattle, followed by Ongole crossbreed cattle and the lowest in Limousin crossbreed cattle. The results of data analysis stated that the breed of cattle had a significant effect ($P<0.05$) on the number of follicles. This may be because Friesian Holstein crossbreed cattle are descendants of Friesian Holstein cattle which are included in the *Bos taurus* breed that live in temperate climates in mainland Europe (Asmarasari *et al.* 2023). Friesian Holstein cattle are known for their high reproductive efficiency with relatively low service per conception and relatively high conception rate (Nasr *et al.* 2021). This may be related to the number of antral follicles they have. According to Morotti *et al.* (2017), in several studies, *Bos taurus* cattle were reported to have a higher antral follicle count (AFC). AFC indicates the number of follicles visualized by ultrasonography in the ovaries. AFC tends to vary in cattle, but has a high repeatability in the same individual. In the last decade, AFC has been considered as a biological marker of fertility with many positive aspects of the reproductive efficiency of animals with high AFC. Animals with high AFC show a greater number and better quality of embryos, better pregnancy rates, increased circulating progesterone concentrations, and other characteristics related to fertility.

During the postpartum period, *Bos taurus* cattle

showed higher LH concentrations than *Bos indicus* cattle. This difference in LH concentration may cause differences in the number of follicles. It is known that Ongole crossbreed cattle are included in *Bos indicus* (Bó *et al.* 2007). Meanwhile, Limousin crossbreed cattle showed fewer follicle results compared to Friesian Holstein crossbreed and Ongole crossbreed cattle, this may be because in Limousin crossbreed cattle there has been poor crossbreeding. This poor crossbreeding causes a decline in reproductive status, from which Limousin cattle as *Bos taurus* cattle should have superior reproductive status.

The Influence of Cow Breed on Oocyte Quality

Figure 1 shows the results of microscopic observation of oocytes. Grade A oocytes (very good) were found, characterized by uniform dark cytoplasm with a round eccentric nucleus and surrounded by a dense cumulus cell layer; Grade B (good) was characterized by uniform dark cytoplasm, some layers of cumulus cells still appeared dense, but less compact; Grade C (moderate) was characterized by less uniform oocytes, the cytoplasm appeared slightly transparent, the oocytes only had a few layers of cumulus cells; Grade D (bad) was characterized by more fragmentation of the cytoplasm, no layer of cumulus cells. much fragmentation of the cytoplasm, no layer of cumulus cells.

Table 3 shows that the number of oocytes from a pair of ovaries belonging to the three breeds of cattle varies. The average number of oocytes was highest in Friesian Holstein crossbreed cattle with grade C oocyte

Table 2. Mean ($\pm$ standard deviation) of cattle breeds for follicle size

Types of cattles	Average number of follicles		
	Small	Medium	Large
Friesian Holstein crossbreed	2.55 $\pm$ 0.63 ^b	1.09 $\pm$ 0.63 ^a	0.604 $\pm$ 0.53 ^a
Ongole crossbreed	2.17 $\pm$ 0.707 ^b	0.89 $\pm$ 0.95 ^a	0.808 $\pm$ 0.708 ^a
Limousine rossbreed	2.39 $\pm$ 0.87 ^b	0.66 $\pm$ 0.76 ^a	0.58 $\pm$ 0.63 ^a

^{a, b} Different superscripts in the same column indicate significantly differences ($P<0.05$)

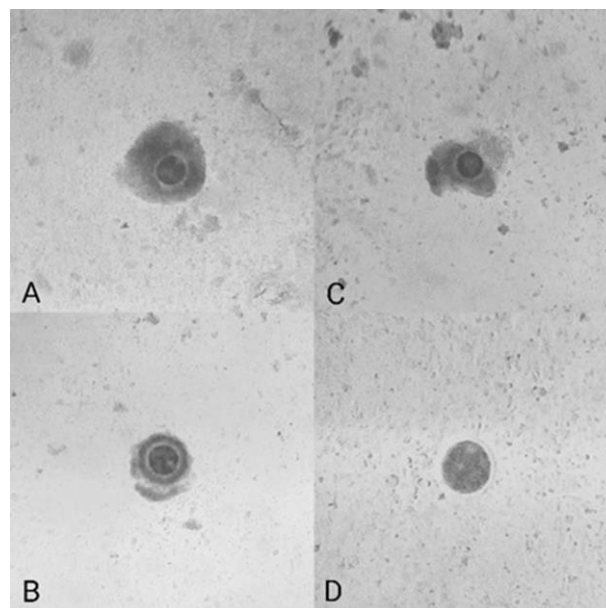


Figure 1. Oocyte quality grade A, B, C, and D with 100x magnification, without staining

quality, which was 1.31 pieces. Based on the results of the analysis of variance, the breed of cattle had a significant effect ($P>0.05$) on the number of oocytes based on oocyte quality and breed of cattle.

Based on oocyte quality, there is a significant difference between the average number of oocytes in grade C, B and A quality. However, there is no significant difference between grade D and B and B and A oocytes. Based on cattle breed, there is a significant difference between Friesian Holstein crossbred cattle and Ongole crossbred cattle. However, there is no significant difference between Friesian Holstein crossbred cattle and Limousine crossbred cattle and Limousine crossbred cattle and Ongole crossbred cattle.

The results showed that the breed of cattle affects the quality of oocytes. Friesian Holstein crossbred cattle had the highest average total number of oocytes. In terms of quality, the oocytes in Friesian Holstein crossbred cattle were mostly grade C. However, Friesian Holstein crossbred cattle also had more grade A and B oocytes compared to Ongole crossbred and Limousine crossbred cattle.

The superior quality of oocytes in Friesian Holstein crossbred cattle can be caused by many factors, one of which is follicular fluid. The follicular fluid surrounding the oocytes in Friesian Holstein cattle has good quality. The follicular fluid is important for the development and maturation of oocytes (Pawliński *et al.* 2023). Friesian Holstein cattle can pass on these traits to Friesian Holstein crossbred cattle as their offspring. Follicular fluid in Friesian Holstein crossbred cattle can have good quality possibly related to metalloproteinase, thrombin, and vitamin D/retinoid X receptor alpha as the main factors affecting the quality of follicular fluid and oocyte quality (Bianchi *et al.* 2016).

The study demonstrates that cattle breed significantly influences oocyte quality and quantity, with Friesian Holstein crossbred cattle exhibiting the highest average number of oocytes, predominantly of

grade C quality, while also producing more grade A and B oocytes compared to Ongole and Limousine crossbreeds. The superior oocyte quality in Friesian Holstein crossbreeds is likely influenced by the composition of their follicular fluid, which plays a crucial role in oocyte development and maturation, potentially due to key factors such as metalloproteinase, thrombin, and vitamin D/retinoid X receptor alpha.

Effect of BCS on Ovarian Weight

The data in Table 4 shows the effect of BCS on ovary weight analyzed using the Chi Square Test. The results of the analysis show that in Friesian Holstein crossbred cattle, BCS affects ovary weight ($P<0.05$) while in Ongole crossbred and Limousine crossbred cattle, BCS affects ovary weight ($P<0.05$). When viewed from the data, it is known that each BCS has a varying ovary weight.

BCS has been shown to significantly impact various aspects of reproductive performance in cattle. Studies consistently show that at the time of mating or breeding, cows with higher BCS are associated with better reproductive outcomes, including better response to estrus induction, higher pregnancy rates, and earlier onset of luteal activity (Cooke *et al.* 2021). However, BCS does not directly impact ovarian weight, consistent with the results of this study.

However, in Friesian Holstein crossbred cattle, the results of this study indicate an effect of BCS on ovary weight. No previous theories or research results have been found that can explain this. In general, BCS has a significant effect on reproductive performance in dairy cattle. Dairy cattle with low BCS tend to show low conception rates (Abdel-Latif 2022).

Follicle activity has been found to affect ovarian weight. Studies have shown that ovaries with more follicles tend to be heavier. This relationship suggests that the presence and development of follicles contribute to overall ovarian size and weight (Dangudubiyam and Ginther 2019). When associated

Table 3. Mean ($\pm$ standard deviation) of cattle breeds on oocyte quality

Types of cattles	Average number of oocytes			
	A	B	C	D
Friesian Holstein crossbred	0.606 $\pm$ 0.57 ^{Ba}	0.85 $\pm$ 0.53 ^{Bab}	1.31 $\pm$ 0.69 ^{Bc}	1.04 $\pm$ 0.52 ^{Bbc}
Ongole Crossbred	0 $\pm$ 0 ^{Aa}	0.65 $\pm$ 0.52 ^{Aab}	1.04 $\pm$ 0.85 ^{Ac}	0.606 $\pm$ 0.6 ^{Abc}
Limousine Crossbred	0.47 $\pm$ 0.59 ^{ABba}	0.403 $\pm$ 0.65 ^{ABab}	1.107 $\pm$ 0.86 ^{ABc}	0.85 $\pm$ 0.69 ^{ABbc}

^{A, B} Different superscripts in the same row indicate significant differences ($P<0.05$)

^{a, b, c} Different superscripts in the same column indicate significant differences ($P<0.05$)

Table 4. Effect of BCS on ovary weight in cattles

Types of cattles	BCS	Ovarian weight		Total
		Below average	Above average	
Friesian Holstein crossbred	Skinny (BCS 2)	26.7%	0%	26.7%
	Medium (BCS 3)	26.7%	20%	46.7%
	Fat (BCS 4)	0%	26.7%	26.7%
Ongole crossbred	Skinny (BCS 2)	0%	6.7%	6.7%
	Medium (BCS 3)	6.7%	33.3%	40%
	Fat (BCS 4)	20%	33.3%	53.3%
Limousine crossbred	Skinny (BCS 2)	0%	13.3%	13.3%
	Medium (BCS 3)	33.3%	13.3%	46.7%
	Fat (BCS 4)	26.7%	13.3%	40%

with this, in Friesian Holstein crossbreed cattle in this study, BCS can affect ovarian weight, perhaps because Friesian Holstein crossbreed cattle in this study showed a higher average number of follicles in cattle with BCS 4 and 3, so indirectly high BCS indicates heavier ovaries because they contain more follicles.

Effect of BCS on Follicle Number

The data in Table 5 shows the effect of BCS on the number of follicles analyzed using the chi square test. The results of the analysis show that BCS in Friesian Holstein crossbreed and Ongole crossbreed cattle affects the number of follicles ($P < 0.05$) while in Limousine crossbreed cattle, BCS does not affect the number of follicles ($P > 0.05$). When viewed from the data, it is known that each BCS has a varying number of follicles.

Low BCS is associated with low insulin-like growth factor 1 (IGF 1). IGF 1 is known to affect gonadotropin releasing hormone (GnRH) by activating the expression of the gonadotropin subunit gene (GTH) which is related to ovarian function (Ando *et al.* 2006). GnRH which is synthesized and released by neuronal cells in the hypothalamus gland then stimulates the anterior pituitary gland to secrete follicle stimulating hormone (FSH) and luteinizing hormone (LH) which are needed for follicle development (Alfons *et al.* 2022). Therefore, BCS can affect the number of follicles through IGF 1 levels.

However, this opinion is different from previous literature theories. Domínguez (1995) stated that BCS does not affect the number of follicles in cows. According to him, the number of follicles is mainly influenced by reproductive status. Pregnant cows show fewer medium and large follicles compared to cyclic (non-pregnant) cows, while the development of small follicles is not inhibited during pregnancy. However, BCS has a significant effect on the number of small

follicles with more follicles in cows with BCS 3 to 5 compared to cows with lower scores.

Effect of BCS on Oocyte Quality

The data in Table 6 shows the effect of BCS on oocyte quality in Friesian Holstein crossbreed, Ongole crossbreed, and Limousine crossbreed cattle analyzed using the Chi Square Test. The results of the analysis show that BCS in Friesian Holstein crossbreed, Ongole crossbreed, and Limousine crossbreed cattle affects oocyte quality ($P < 0.05$). The data show that oocyte quality varies across different BCS categories.

BCS affects oocyte quality in cattle. Oocyte quality is greatly influenced by the nutritional status and overall health of the cattle, as reflected in its BCS (Velazquez 2023). Specifically, previous studies have found that cattle with optimal BCS (BCS 3) tend to have better oocyte quality, with a higher proportion of embryo and blastocyst divisions than cattle with lower (BCS 1 and 2) or higher (BCS 4 and 5) BCS (Bezdiček *et al.* 2020).

Cows with optimal BCS tend to have better nutritional status, which is associated with improved oocyte quality. As explained by Velazquez (2023), hormones in the cow's body, especially estrogen and progesterone levels, can be influenced by BCS. Optimal BCS has a better metabolic and hormonal microenvironment, thus conducive to oocyte development. This metabolic microenvironment includes factors such as chemical composition, pH, and glycolysis concentration that affect the synthesis and secretion of hormones such as estrogen and progesterone (Gu *et al.* 2015). In addition, cow BCS also affects the dynamics of ovarian follicle development, which is very important for oocyte quality. Cows with optimal BCS tend to have better follicles, characterized by a greater proportion of large follicles (Velazquez 2023).

Table 5. Effect of BCS on follicle number in cattles

Types of cattles	BCS	Percentage of number of follicles			Total
		Small	Medium	Large	
Friesian Holstein crossbreed	Skinny (BCS 2)	12.87%	3.30%	1.65%	17.82%
	Medium (BCS 3)	25.41%	6.60%	4.29%	36.3%
	Fat (BCS 4)	40.59%	2.31%	2.97%	45.87%
Ongole crossbreed	Skinny (BCS 2)	4.62%	0.33%	0%	4.95%
	Medium (BCS 3)	20.79%	2.64%	2.97%	26.40%
	Fat (BCS 4)	28.71%	11.22%	5.28%	45.21%
Limousine crossbreed	Skinny (BCS 2)	6.93%	0.33%	0.33%	7.59%
	Medium (BCS 3)	38.28%	6.93%	2.31%	47.52%
	Fat (BCS 4)	21.78%	2.31%	2.97%	27.06%

Table 6. The Effect of BCS on oocyte quality in cattles

Types of cattles	BCS	Oocyte grade				Total
		A	B	C	D	
Friesian Holstein crossbreed	Skinny (BCS 2)	1.42%	1.42%	4.96%	8.51%	16.31%
	Medium (BCS 3)	4.26%	8.51%	23.40%	14.89%	51.06%
	Fat (BCS 4)	6.38%	10.64%	14.18%	1.42%	32.62%
Ongole crossbreed	Skinny (BCS 2)	0%	0%	0%	4.67%	4.67%
	Medium (BCS 3)	1.87%	8.41%	14.95%	8.41%	33.64%
	Fat (BCS 4)	2.8%	15.89%	31.78%	11.21%	61.68%
Limousine crossbreed	Skinny (BCS 2)	0.73%	0.73%	0.73%	2.19%	4.38%
	Medium (BCS 3)	5.11%	5.11%	27.74%	13.87%	51.82%
	Fat (BCS 4)	9.49%	10.95%	13.87%	9.49%	43.8%

The results of this study showed that BCS 3 and 4 had the most oocytes with quality C. Although oocytes with quality C have incomplete cumulus cell layers, Moreno *et al.* (1992) have tried to use these oocytes for IVM and IVF, and some of these oocytes can still mature, be fertilized and develop into embryos. This suggests that some oocytes may lose some of their cumulus cells during the aspiration and handling process without losing viability, which is the ability of the oocyte to maintain its function.

CONCLUSION

The breed of cattle does not affect the weight of the ovary, but affects the number of follicles, and the quality of oocytes. In Friesian Holstein crossbreed cattle, BCS affects the weight of the ovary, the number of follicles, and the quality of oocytes. In Ongole crossbreed cattle, BCS does not affect the weight of the ovary, but affects the number of follicles and the quality of oocytes. In Limousine crossbreed cattle, BCS does not affect the weight of the ovary and the number of follicles, but affects the quality of oocytes.

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REFERENCES

- Abdel-Latif FH. 2022. Body Condition Score and its association with productive and reproductive performance and health status in dairy cattle: Review article. *IOP Conference Series: Earth and Environmental Science*, 1060(1):012069. 10.1088/1755-1315/1060/1/012069.
- Aguila L, Treulen F, Therrien J, Felmer R, Valdivia M, Smith LC. 2020. Oocyte selection for in vitro embryo production in bovine species: Noninvasive approaches for new challenges of oocyte competence. *Animals*, 10(12):2196. Doi: 10.3390/ani10122196.
- Alfons MPW, Budiyo A, Setyawan EMN. 2022. Kajian profil hormon estradiol berdasarkan perkembangan folikel dan ovarium sapi potong postpartus. *Jurnal Sain Veteriner*, 40(1):24-31.
- Ando H, Luo Q, Koide N, Okada H, Urano A. 2006. Effects of insulin-like growth factor I on GnRH-induced gonadotropin subunit gene expressions in masu salmon pituitary cells at different stages of sexual maturation. *General and Comparative Endocrinology*, 149(1):21-29.
- Araújo VR, Gastal MO, Figueiredo JR, Gastal EL. 2014. In vitro culture of bovine preantral follicles: A review. *Reproductive Biology and Endocrinology*, 12(1):78. <https://doi.org/10.1186/1477-7827-12-78>.
- Asmarasari SA, Azizah N, Sutikno S, Puastuti W, Amir A, Praharani L, Rusdiana S, Hidayat C, Hafid A, Kusumaningrum DA, Saputra F, Talib C, Herliatika A, Shiddieqy MI, Hayanti SY. 2023. A review of dairy cattle heat stress mitigation in Indonesia. *Veterinary World*, 16(5):1098-1108.
- Berry DP, Buckley F, Dillon P, Evans RD, Rath M, Veerkamp RF. 2003. Genetic relationships among body condition score, body weight, milk yield, and fertility in dairy cows. *Journal of Dairy Science*, 86(6):2193-2204.
- Bezdiček J, Nesvadbová A, Makarevich A, Kubovičová E. 2020. Relationship between the animal body condition and reproduction: The biotechnological aspects. *Archives Animal Breeding*, 63(1):203-209.
- Bianchi L, Gagliardi A, Landi C, Focarelli R, Leo V, Luddi A, Bini L, Piomboni P. 2016. Protein pathways working in human follicular fluid: The future for tailored IVF?. *Expert Reviews in Molecular Medicine*, 18(9):1-14.
- Bó GA, Cutaia L, Peres LC, Pincinato D, Maraña D, Baruselli PS. 2007. Technologies for fixed-time artificial insemination and their influence on reproductive performance of *Bos indicus* cattle. *Society of Reproduction and Fertility Supplement*, 64(1):223-236.
- Chatiza FP, Bartels P, Nedambale TL, Wagenaar GM. 2013. Sperm-egg interaction and functional assessment of springbok, impala and blesbok cauda epididymal spermatozoa using a domestic cattle in vitro fertilization system. *Animal Reproduction Science*, 143(1-4):8-18.
- Cooke RF, Lamb GC, Vasconcelos JLM, Pohler KG. 2021. Effects of body condition score at initiation of the breeding season on reproductive performance and overall productivity of *Bos taurus* and *B. indicus* beef cows. *Animal Reproduction Science*, 232(1):106820. Doi: 10.1016/j.anireprosci.2021.106820.
- Currin L, Baldassarre H, Bordignon V. 2021. In vitro production of embryos from prepubertal Holstein cattle and Mediterranean water buffalo: Problems, progress and potential. *Animals (Basel)*, 11(8):2275. Doi: 10.3390/ani11082275.
- Dangdubiyyam SV, Ginther OJ. 2019. Relationship between more follicles in right than left ovary in recently born calves and right ovary propensity for ovulation in cattle. *Reproductive Biology*, 19(4):363-367.
- Domínguez MM. 1995. Effects of body condition, reproductive status and breed on follicular population and oocyte quality in cows. *Theriogenology*, 43(8):1405-1418.
- Dorice AK, Ferdinand N, Justin K, Augustave K, Linda KK. 2019. Effect of breed, age, body condition score, and nutritional status on follicular population, oocyte yield, and quality in three Cameroonian Zebu cattle *Bos indicus*. *Hindawi Advances in Agriculture*, 2019(1):1-15.
- Ferré LB, Kjelland ME, Strøbech LB, Hyttel P, Mermillod P, Ross PJ. 2020. Review: Recent advances in bovine in vitro embryo production: Reproductive biotechnology history and methods. *Animal*, 14(5):991-1004.
- Gu L, Liu H, Gu X, Boots C, Moley KH, Wang Q. 2015. Metabolic control of oocyte development: Linking maternal nutrition and reproductive outcomes. *Cellular and Molecular Life Sciences*, 72(2):251-271.
- Handarini R, Sudrajat D, Hardiansyah D. 2014. Kualitas oosit dari ovarium sapi peranakan Ongole (PO) pada fase folikuler dan luteal. *Jurnal Pertanian*, 5(2):89-94.
- He M, Zhang T, Yang Y, Wang C. 2021. Mechanisms of oocyte maturation and related epigenetic regulation. *Frontiers in Cell and Developmental Biology*, 9(1):654028. Doi: 10.3389/fcell.2021.654028.
- Mahzabin R, Khandoker MAMY, Husain SS, Islam MR, Shathi SJ, Habib MR, Vargas-Bello-Pérez E. 2020. Evaluación de ovarios y folículos bovinos mediante análisis histológico para la producción potencial in vitro de embriones en condiciones tropicales. *Tropical and Subtropical Agroecosystems*, 23(3):1-9.
- Moreno JF, Turczynski CJ, Foxworth GF, Kraemer DC. 1992. Influence of parity of donor and presence of a cl on quantity and quality of bovine oocytes from ovarian follicles a spirated post mortem. *Biology of Reproduction*, 48(1):169 (Abstr).
- Morotti F, Zangirolamo AF, da Silva NC, da Silva CB, Rosa CO, Seneda MM. 2017. Antral follicle count in cattle: Advantages, challenges, and controversy. *Animal Reproduction*, 14(3):514-520.
- Murasawa M, Takahashi T, Nishimoto H, Yamamoto S, Hamano S, Tetsuka M. 2005. Relationship between ovarian weight and follicular population in heifers. *The Journal of Reproduction and Development*, 51(5):689-693.
- Nasr MAF, Hussein MA, Alkhedaide AQ, El-Tarabany MS, Roushdy EM. 2021. Reproductive performance and culling rate of purebred Holstein cows and their crosses with Fleckvieh and Brown Swiss cows under subtropical conditions. *Frontiers in Veterinary Science*, 8(1):752941. Doi: 10.3389/fvets.2021.752941.
- Nielsen SS, Sandøe P, Kjølsted SU, Agerholm JS. 2019. Slaughter of pregnant cattle in Denmark: Prevalence, gestational age, and reasons. *Animals (Basel)*, 9(7):392.
- Pawliński B, Petrajtis-Golobów M, Trela M, Witkowska-Piłaszewicz O. 2023. Acid-base, gas, ions, and glucose analysis in follicular fluid in Holstein-Friesian dairy cows is associated with the follicle size in Poland. *Animals (Basel)*, 13(10):1636. <https://doi.org/10.3390/ani13101636>.

- Rabiee AR, Lean IJ, Gooden JM, Miller BG. 1999. Relationships among metabolites influencing ovarian function in the dairy cow. *Journal of Dairy Science*, 82(1):39-44.
- Shari A. 2022. Seleksi spermatozoa pada in vitro fertilization (IVF). *Indonesian Journal of Health Science*, 2(1):1-8.
- Syaiful FL. 2021. Morfometri ovarium dan folikel sapi lokal sebagai penghasil oosit untuk in vitro fertilization. *Jurnal Embrio*, 13(2):57-64.
- Velazquez MA. 2023. Nutritional strategies to promote bovine oocyte quality for in vitro embryo production: Do they really work. *Veterinary Sciences*, 10(10):604. <https://doi.org/10.3390/vetsci10100604>.