DESCRIPTION OF GRAAFIAN FOLLICLES IN A SIMENTAL COW EXPERIENCED IN A CASE OF DELAYED OVULATION

Langgeng Priyanto[1*], Herdis Herdis[2], Agung Budianto[3], Chandra Brahmantya[4*], Erma Safitri[5], Santoso Santoso[2], Rahma Isartina Anwar[2], Tri Puji Priyatno[2], Pradita Iustitia Sitaresmi[2], Suhesti Hartati[6], Arfan Abrar[1], Aptriansyah Susanda Nurdin[1], Ahmad Falahul Irfan[1]

[1]Department of Animal Science, Faculty of Agriculture, Sriwijaya University, South Sumatra, 30862, Indonesia;

[2]Research Center for Animal Husbandry, National Research and Innovation Agency, Cibinong Science Center, Jalan Raya Jakarta-Bogor, Bogor, 16915, Indonesia;

[3]Fakultas Kedokteran Hewan. Universitas Gadjah Mada. Yogyakarta. Indonesia;

[4]Reproductive Biology Master Student of Faculty of Veterinary Medicine Airlangga University;

[5]Division of Veterinary Reproductive, Department of Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, East Java, 60115, Indonesia;

[6]Sembawa Center for Superior Animal Breeding and Forage, Directorate General of Animal Husbandry and Animal Health, Ministry of Agriculture, Indonesia.

[1] langgengpriyanto@fp.unsri.ac.id (First Author, ORCHID: 0000-0001-6776-664x)*

[4] drh.chandrabrahmantya@gmail.com (Corresponding author, ORCID: 0009-0009-0123-0397)*

Abstract

The purpose of this study was to look at the ovarian follicular dynamics in crossbreed cattle diagnosed with delayed ovulation. The cattle used were 2 crossbreed cattle, 1 of which was diagnosed as having delayed ovulation with the Simmental type and 1 normal cow with the Ongole breed. Observation of ovarian follicular dynamics was carried out according to the Fricke method with repeated scanning of the ovarian surface to obtain an overview of the follicles. Parameters observed were the number and diameter of the follicles in the ovaries of cows which were measured using an internal clipper on ultrasound, namely the distance between the two axis points based on the longest axis with units of cm, to determine development during one estrus cycle. The results showed that simmental crossbreed cattle that experienced delayed ovulation in the table had a follicle de graaf size of 1.5, this size was smaller compared to ongole cross-breed cattle with a size of 2 CM. Based on the results of the study it can be concluded that the difference in follicle size has no effect on the case of delayed ovulation, cows that experience ovulation delay experience prolonged estrus and ultrasound results have been obtained in cattle that experience ovulation delay that ovulation occurs on the 3rd day of estrus to be precise when the cow was at the end of estrus

Keywords: Delayed ovulation, Follicles and Crossbreed Cows

I. INTRODUCTION

Farmers prefer crossbreed cattle because the size of the cattle is larger, so the selling price of cattle is much higher compared to local cattle, but in fact, the availability of domestic beef is not enough to meet national consumption needs. Bull semen quality and reproduction can fluctuate throughout the year, and this is apparent in both temperate and tropical climates, owing to factors such as genotype, environment, and linkages between the two (Priyanto et al. 2023a). The successful use of sexed sperm in bovines has been documented; the most common application of sexed sperm is for the sex preselection of bulls to achieve an adequate number of national beef cattle (Priyanto et al. 2023b). This insignificant increase in population numbers is partly influenced by the large number of cows experiencing diseases such as reproductive disorders. The most recent case of reproductive disorders in crossbred cattle is delayed ovulation. Cases of delayed ovulation are caused by low levels of the hormone luteinizing hormone (LH) in the blood. Low LH levels cause the follicular phase to be prolonged, so that the follicles that should ovulate and enter the luteal phase are delayed or do not occur at all, which can cause reproductive inefficiency in people's livestock. Reproductive inefficiency is the problem most often experienced by small-holder livestock. The cause of reproductive inefficiency is decreased fertility. One of the causes of decreased fertility is suboptimal follicle size and cases of delayed ovulation in cows.

Delayed ovulation is a reproductive disorder in livestock that causes a prolonged estrus period, namely more than 36 to 48 hours before ovulation occurs, has a normal estrus cycle, and has no abnormalities in the estrus mucus or reproductive tract (Honparkhe et al. 2010). The size of the ovaries and follicles varies greatly between individuals, even at the same age (Mossa et al., 2012). According to Keskin et al. (2016), female cows with high pregnancy rates have small follicle sizes; this is in contrast to the opinion of Perry et al. (2005), who stated that cows with high gestation have large follicle sizes. There are differences of opinion from previous research; this makes the variation in the size of the ovaries and follicles very high in the good reproductive performance of cows, including in Indonesia. Dissent. Decreased cow fertility affects reproductive performance. Based on the explanation above, research will be carried out with the title Description of the Development of DeGraaf Follicles in Cows Experiencing Delayed Ovulation Cases using Ultrasound.

II. METHODS

Experimental animals

This research was carried out from October to December 2022 in East Ogan Komering Ulu Regency and at BPTU HPT Sembawa Banyuasin Regency, South Sumatra. Selection of Experimental Animals The cows used were 2 crossbreed cows, namely 1 cow that was diagnosed as having delayed ovulation of the Simmental type and 1 normal cow of the Ongole crossbreed type.

The cow is clinically healthy (aged 2–8 years; parity 1-4; body condition score (BCS) 3.0–4.0 on a scale of 1–5), has a normal estrus cycle, and has no abnormalities in the estrous mucus and reproductive tract (Honparkhe et al. 2010).

Observations Using Ultrasonography Observations were made once every day during one estrus cycle until ovulation occurred in the next cycle to get a clear picture of ovarian follicular dynamics. (Sukareksi 2019).

Estrus Observation

Observation of estrus is carried out visually every day, from before it is detected until it stops. The focus of attention during estrus observation is the duration of the estrus and the intensity of the estrus. The duration of estrus is the interval (distance) calculated from the first appearance of estrus symptoms until the cessation of estrus symptoms in units (hours), while the intensity of estrus includes the physical changes that occur in the cow's external reproductive organs. The intensity of estrus is observed based on the method (Listian, 2005) by giving a score based on the signs of estrus that appear in livestock, namely : vulva changes, cervical mucus, behavior, and the degree of uterine tension (Table. 1).

Table. 1 The Signs of Estrus Scoring

Scoring	**Vulva Changes Appearance**	**Cervical Mucus**	**Behaviour**	**Degree of Uterine Tension**
1	The vulva is pink, and peripheral blood vessels are not clearly visible.	Mucus is transparent, small in quantity, and hangs from the vulva	No changes in behavior	Weak
2	The vulva is reddish, and peripheral blood vessels are clearly visible.	Mucus is transparent, quite large in quantity, and hangs from the vulva	Showing one behavioral symptom only	Medium
3	Dark red, visible branching of peripheral blood vessels	Transparent mucus, abundant, is seen hanging from the vulva, around the base of the tail, and the floor	Showing two or more then behavioral symptom	Stiff

Observed parameters

The parameters observed were the number and diameter of follicles in the cow's ovaries, which were measured using an internal clipper on ultrasound, namely the distance between the two axis points based on the longest axis in cm units. (Lucy et al., 1992). Follicles are grouped into small follicles with a diameter of 0.3 to 0.5 cm (Class I), medium follicles with a diameter of 0.6 to 0.9 cm (Class II), and large follicles >1 cm (Class III) to determine development during one estrous cycle (21 days).

Data analysis

The qualitative data obtained will be analyzed descriptively and presented in table form.

III. RESULTS AND DISCUSSION

Estrus Intensity

Observation of estrus intensity was carried out visually starting from before estrus at the beginning of the first estrus cycle until the next estrus. Observations are carried out every day which are divided into 2 parts, namely morning and evening observations. Determining the value of lust behavior is done by observing changes in the behavior of female animals. The behavioral intensity value is the sum of all behavioral values that appear, such as restlessness, mooing and climbing on a friend, shown by the cow (Listiani, 2005).

Table 2. Results of the average estrus intensity score of simmental crossbreed and ongole crossbreed cows

Estrus Signs	Simmental	Ongole crossbreed
Behaviour	3	3
Cervical Mucous	3	3
Vulva Changes	2	2
Degree of Uterine Tension	3	3

Sources. 2022 Research Data

Estrus intensity score, which includes changes in behavior, abundance of cervical mucus, changes in vulva color, and degree of uterine tension. The estrus intensity scores for both cows were classified as good enough, as seen from the clear signs of estrus through the estrus intensity scores. This is in line with the findings of Lyimo et al. (2000) 39% of estrus intensity in cows is influenced by increasing levels of the hormone estrogen during estrus. Determining the value of lust behavior is done by observing changes in the behavior of female animals. The behavioral intensity value is the sum of all behavioral values that appear, such as restlessness, mooing, and climbing on a friend, as shown by the cow (Listiani, 2005).

Cycle and Duration of Estrus

Estrus duration is the estrus interval calculated from the first time the cow shows symptoms of estrus until the absence of dominant follicles which can be observed using ultrasound. estrus until the symptoms of estrus disappear.

Table. 3 Results of the estrus cycle of both types of cows

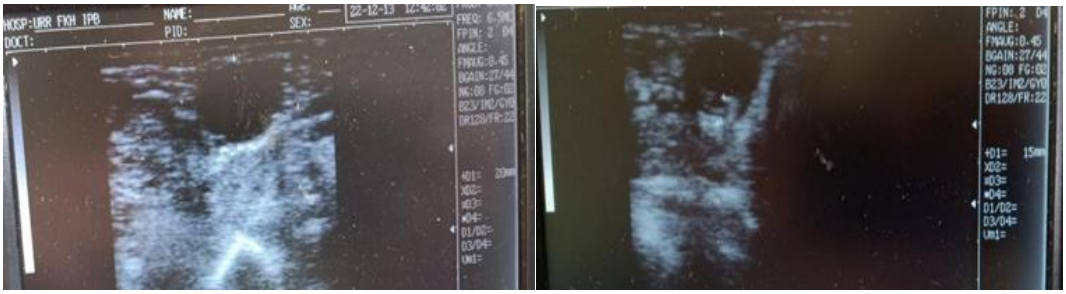
Breed	Estrus Cycle (Day)	Duration of estrus (hour)	Ovulation (hour)
Simmental	23	48	48
Ongole Crossbreed	21	24	12

Sources. 2022 Research Data

The estrus cycle in simmental crossbreed cows experiences delayed ovulation in Table 3, around 22 days, and in Ongole crossbreed cows, 21 days, which is still categorized as normal. Simmental cows that experience delayed ovulation experience a long estrus duration of up to 48 hours; this is in accordance with the statement (Dadarwal et al., 2005). 30% of cases of delayed ovulation in crossbred cows occur in cows with an estrus duration of 37–60 hours, and 40% occur in cows with an estrus duration of 24-36 hours. The duration of estrus for Ongole crossbreed cows is 24 hours; the time of estrus varies between 18 and 19 hours (Hafez, 2000).

Graaf Follicle Observation

De Graaf follicles are the final and largest form of tertiary follicles. This phase is the process of determining or selecting a dominant follicle that will ovulate. The growth and development of follicles reaches its peak in the form of mature Graafian follicles and ovulation only occurs in non-pregnant animals after puberty during a reproductive cycle (Feradis, 2010).



a b

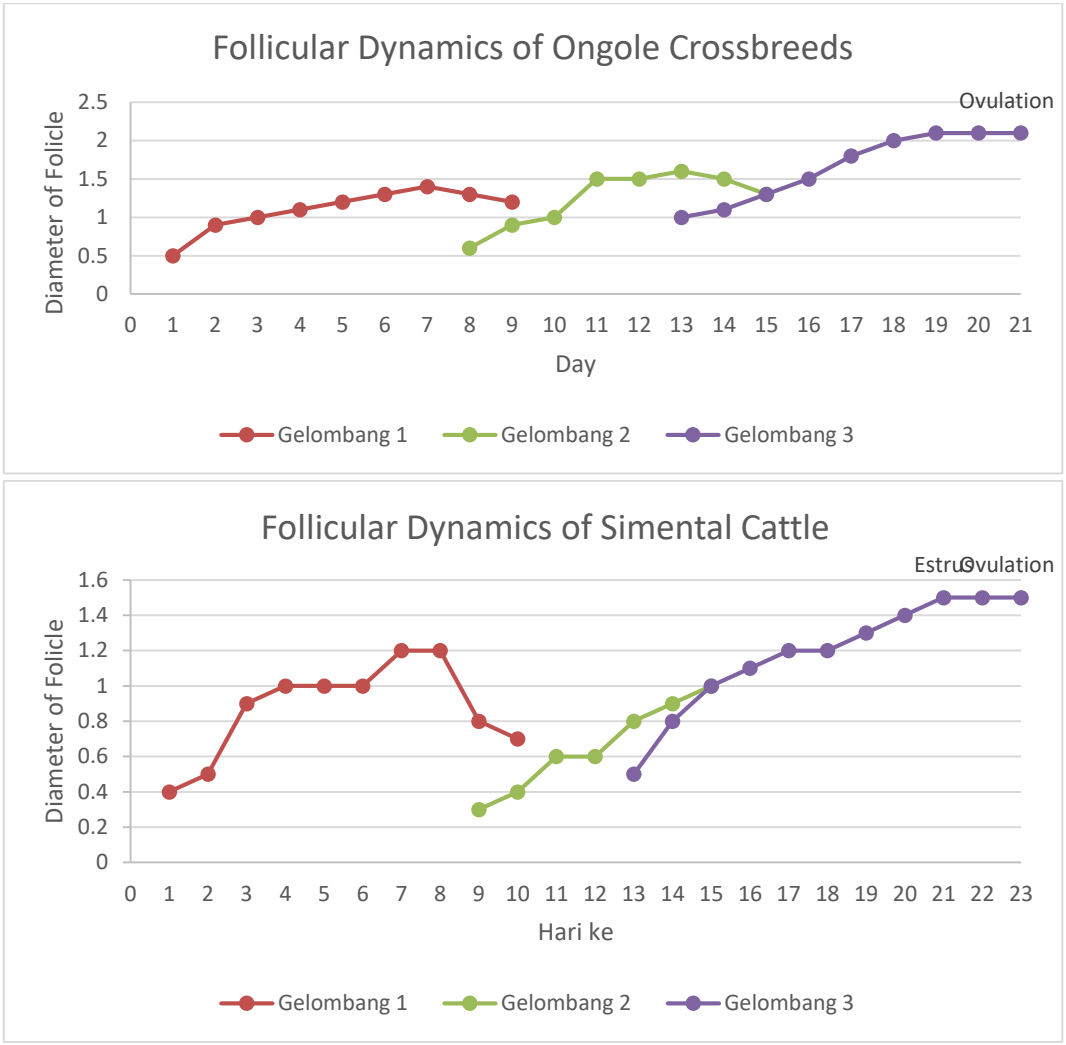
Figure 1. (a) Ultrasound image of degraaf follicles in Ongole crossbreed cattle; (b) Ultrasound image of degraaf follicles in Simmental cattle

Simmental crossbreed cows that experience delayed ovulation in the table have a degraaf follicle size of 1.5; this size is smaller compared to Ongole crossbreed cows with a size of 2 CM. The size of the ovaries and follicles varies greatly between individuals, even at the same age (Mossa et al., 2012). According to Keskin et al. (2016), female cows with high pregnancy rates have small follicle sizes; this is in contrast to the opinion of Perry et al. (2005), who stated that cows with high gestation have large follicle sizes. There are differences of opinion from previous research; this makes the variation in the size of the ovaries and follicles very high in the good reproductive performance of cows, including in Indonesia.

Ovarian Dynamics

Follicular dynamics in animals occurs in the form of waves of follicular development. A wave of follicular development includes the simultaneous growth of a group of follicles, one of which will become the dominant follicle, reaching its largest size, and will suppress the development of other smaller follicles (Siregar, 2010).

Figure 2. Follicular Dynamics of Simental Cattle and Ongole Crossbreeds



Sources. 2022 Research Data

According to the average diameter of follicles during one estrus cycle in simmental crossbreed cows that experience delayed ovulation using ultrasonography, there are three follicle wave patterns that occur during one estrus cycle. Figure 2 shows that in delayed ovulation cows, the growth of follicle waves is normal. Ongole crossbreed cattle also have three normal follicular wave patterns. Ovulation in Simmental crossbreed cows that experience delayed ovulation occurs on the 3rd day of estrus. This shows that there is an obstacle to the LH surge in delayed ovulation cows, which causes a delay in ovulation time, whereas in Ongole crossbreed cows, ovulation occurs on the 2nd day of estrus. Hafez (2000) reported that the duration of estrus in cows is 18–19 hours and ovulation 10–11 hours after estrus. Follicular development is characterized by waves of follicular growth. One wave is defined as a process of synchronous follicular growth from several small follicles. From this group of small follicles, one of them will be selected and grow into a dominant follicle, while the other follicles will stop growing and go into resistance.

After reaching its maximum size, the dominant follicle will also experience pressure and regression. Atpression of the dominant follicle will cause the growth of a wave of new follicles. During the estrus cycle period, there are 2–3 waves of follicles. In the second wave, the dominant follicle will become an anovulatory follicle, while the dominant follicle from the third wave will ovulate.

IV. CONCLUSION

There are differences in the size of the follicles, especially in the size of the de Graaf follicles, but the difference in follicle size has no effect on cases of delayed ovulation, cows that experience delayed ovulation experience prolonged estrus and ultrasound results have been obtained on delayed ovulation cows that ovulation occurs on the 3rd day of estrus, to be precise. when the cow is at the end of estrus.

V. ACKNOWLEDGMENTS

Thank you to Sriwijaya University for allowing us to take part in the post-doctoral program at National Research and Innovation Agency (BRIN), and thank you to BRIN for giving us the opportunity to take part in the post-doctoral program.

VI. REFERENCES

Dadarwal D, Singh J, Honparkhe M, Cheede G S and Kang R S. 2005.

Feradis, A. 2010. Teknologi Reproduksi ternak. Alfabeta, Bandung. Investigations On Repeat Breeding Crossbred Cattle with History of *Delayed ovulasi*. *Indian Journal of Animal Science* 75 (8): 922– 24.

Hafez, E.S.E, 1993, Reproduction Failure in Females, 6 th Edition, LEA And Febiger, Philadelphia, pp: 267, 271

Honparkhe M, Singh J, Dadarwal D, Ghuman S P S, Dhaliwal G S and Kumar A. 2010. Effect Of Midluteal Phase GnRH Treatment in Repeat Breeder Cattle. *Indian Veterinary Journal* 87: 351–54.

Keskin, A., Mecitoglu, G., Bilen, E., Guner, B. 2016. The Effect of Ovulatory Follicle Size at The Time Of Insemination on Pregnancy Rate in Lactating Dairy Cows. Turkish Journal Veterinary and Animal Sciences. 40: 68-74

Listiani D. 2005. Pemberian PGF2a Pada Sapi Peranakan Ongole Yang Mengalami Gangguan Korpus Luteum Persisten. [tesis]. Semarang: Universitas Diponegoro

Lucy MC, Savio JD, Badinga L, De La Sota LR, Thatcher WW. 1992. Factor That Affect Ovarian Follicular Dynamics in Cattle. *J. Anim Sci.* 70: 3615-3626.

Lyimo, Z.C., M. Nielen, W. Ouweltjes, T.A.M. Kruip, and F.J.C.M. van Eerdenburg. 2000. Relationship among estradiol, cortisol and intensity of estrous behavior in dairy cattle. Theriogenology. 53:1783-1795.

Mossa, F., Walsh, S. W., Butler S. T., Berry, D. P., Carter, F., Lonergan, P., Smith, G. W., Ireland, J. J., dan Evans, A. C. O. 2012. Low Number of Ovarian Follicles ≥ 3mm in Diameter are Associated With Low Fertility in Dairy Cows. Jounal Dairy Science. 95: 2355-2361

Perry, G., 2004, “The Bovine Estrous Cycle”, Extension Beef Reproduction Management Specialist, South Dakota State University, Cooperrative Extension Service, USDA, FS921A

Priyanto, L., Herdis, ., Santoso S., Sitaresmi, P. I., Priyatno, T., Anwar, R., Mahari, D., Bety, F., Lupitasari, I., Surachman, M., Wayan, I., Darmawan, A., Abrar, A., Setiawan, A. 2023a. The Effects of Various High Dosage of α- Tocopherol and Ascorbic Acid in Tris Egg Yolk Extender on Post-Thawed Sperm Quality in Tropical Brahman Bulls. Pakistan Journal of Zoology. 10.17582/journal.pjz/20230131020144.

Priyanto L, Herdis H, Santoso S, Anwar RI, Priyatno TP, Sitaresmi PI, Azhari F, Gunawan M, and Putranti OD. 2023b. The reproductive success of Simmental bovine after sex-sorting under various incubation and centrifugation protocols, *Veterinary World*, 16(3): 631–637.

Siregar, G. 2012. Analisis Kelayakan dan Strategi Pengembangan Usaha Ternak Sapi potong. Agrium, 17(3), 192–201.