American Journal of Animal and Veterinary Sciences

Original Research Paper

Pregnancy Rate and Reproductive Disorders Examination of Inseminated Brahman Cross Cows by Rectal Palpation and Ultrasonography

[1,2]Mohamad Ervandi, [3]Muhammad Nur Ihsan, [3]Sri Wahjuningsih and [3]Trinil Susilawati

[1]Faculty of Animal Science, University of Brawijaya, Malang, Indonesia
[2]Faculty of Agricultural Science, Muhammadiyah University of Gorontalo, Gorontalo, Indonesia
[3]Department of Animal Production, Faculty of Animal Science, University of Brawijaya, Malang, Indonesia

Article history
Received: 14-12-2019
Revised: 02-03-2020
Accepted: 27-03-2020

Corresponding Author:
Mohamad Ervandi
Faculty of Agricultural Science, Muhammadiyah University of Gorontalo, Gorontalo 96181, Indonesia
Email: ervandi_husain@yahoo.co.id

Abstract: Pregnancy is a condition when a fetus began to develop in the uterus of the cow. Early pregnancy detection becomes important as it would determine the success of breeding management as well as to achieve the best productivity. This study aimed to evaluate and observe the pregnancy rate and reproductive disorders of Brahman Cross (BX) cows and compare the results to the Ultrasonography (USG) observation. The materials of the study were 80 BX cows selected through a purposive sampling method with the following criteria: aged 2-3 years, weighed 300-500 kg, has at least calved once and had the body condition score (BCS) at 4-7 (in 1-9 scale). This study was conducted as experimental research, with 40 BX cows were artificially inseminated with Ongole crossbred (PO) semen at 0-2 h interval and the other 40 BX cows were at 8-10 h interval from the estrous detection. All of the BX cows were firstly synchronized with PGF2α (LUTALYSE) and then inseminated with PO semen (>40% motility) as according to the Indonesian standard. The pregnancy rate of the BX cows was observed through rectal palpation and 17β-estradiol hormone observation and further examined for the reproductive disorders by using USG. The obtained data were then analyzed descriptively and the results showed that 0-2 h insemination had a higher success of pregnancy (32.5%) compared to the 8-10 h interval (27.5%). Moreover, the highest 17β-estradiol in the inseminated BX cows was at 5,765 ng/ml, while the lowest was at is 0.41 ng/ml. The USG observation showed that 6 BX cows (7%) in this study had a follicular cyst, 2 BX cows (2.25%) had persistent corpus luteum and 1 BX cows (1.25%) had ovarian hypofunction.

Keywords: Brahman Cross Cows, Reproductive Disorders, Rectal Palpation, Ultrasonography, 17β-Estradiol

Introduction

Beef cattle have been the main meat producers and source of the nutritional animal-based protein. The demand for beef consumption in Indonesia has been increasing along with the increased human population and could not be fulfilled solely from the local breeds. The breeding of local cattle with other breeds with higher meat production provides an alternative way to fulfill the demand for meat consumption. Brahman Cross (BX) cattle is one of the crossbreed cattle developed in Indonesia aimed to achieve the goal. The BX cattle had shown good adaptability to the tropical climates, survivability to ectoparasites, as well as good body weight gain and productivity even fed with a low quality feed. The production performance of BX cattle, thus showed its potential to be reared in Indonesia in order to increase domestic meat production (Kuswati and Susilawati, 2016).

The common breeding practice in Indonesia is by natural breeding or Artificial Insemination (AI). The AI has shown many beneficiaries compared to the natural breeding, however, the success of AI would be determined by several factors such as heat detection, inseminating skills and the body condition of the cows (Saacke, 2008; Roelofs *et al*., 2010; Susilawati, 2011). Silent heat is commonly cases found on the farm which

will prevent farmers to make the correct decision to determine the time for artificial insemination. Yekti *et al.* (2017) described that silent heat is the condition where cows did not express clear signs of heat due to the low estrogen level.

Pregnancy is defined as the post-mating physiological period that resulted in the conception and then followed with embryo development into a fetus until parturition (Hafez and Hafez, 2000). Various pregnancy diagnosis can be done depending on the reproductive anatomy and physiological condition of the livestock. In large livestock, such as cows, the most practical and reliable diagnosis is by detecting rectal palpation or by using Ultrasonography (USG) (Fricke, 2005).

The current conditions showed that the AI of BX cows still had the low success of pregnancy. Research by Annashru *et al*. (2017) showed that different time of AI would affect the calving to non-return rate of BX cows, with 0-4 h insemination interval from detected estrous was higher (70%) compared to the 8-12 h (37.14%). The condition thus showed that the time interval of insemination could affect the success of AI as well. In this study, we observe the different time intervals of insemination to the pregnancy rate and occurred reproductive disorders in BX cows.

Materials and Methods

Materials

The materials of this study were 80 Brahman Cross (BX) cows selected through a purposive sampling method with the following criteria: aged 2-3 years, weighed 300-500 kg, have at least calved once and had the body condition score (BCS) at 4-7 (in 1-9 scale).

Methods

This study was conducted as experimental research. All of the BX cows were firstly synchronized with PGF2α (LUTALYSE) and then inseminated with PO semen (>40% motility) as according to the Indonesia standard. The first treatment (P1) were 40 BX cows inseminated at 0-2 h intervals from estrous detection, while the second treatment (P2) were 40 BX cows inseminated at 8-10 h intervals from estrous detection. The pregnancy rate of the BX cows was observed through rectal palpation at 90-days after insemination, while the 17β-estradiol hormone measurement was done by firstly taking the blood sample of BX cows from the jugular vein in the tail area at 90-days after insemination. The sample was then measured for the 17β-estradiol hormone by using commercial ELISA kit (Bioassay Tecnology Laboratory, Shanghai) according to the manufacturer's instruction: http://www.bt-laboratory.com/product/bovine-estrogen-elisa-kit-2/. The USG (Mindray DP-50) observation was done by following Royal *et al*. (2000). The pregnancy rate was analyzed descriptively and formulated as follows:

$$\frac{Total\ pregnant\ BX\ cows}{Total\ performed\ insemination} \times 100\%$$

The Non-Return Rate (NRR) of the inseminated BX cows were also observed frequently at 18-days (NRR_1), 36-days (NRR_2) and 54-days (NRR_3) after insemination to detect early failed pregnancy.

Results

The Pregnancy Rate of the Brahman Cross Cows

The pregnancy observation by using rectal palpation of BX cows in this study is presented in Table 1.

The results showed that the overall successful pregnancy of artificially inseminated BX cows both at P1 and P2 was 30%. Furthermore, the pregnancy rate at P1 or 0-2 h insemination interval (32.5%) was higher compared to the P2 or 8-10 h interval (27.5%). The low successful pregnancy of the BX cows was caused by disorders in the ovary, especially ovarian hypofunction, follicular cyst, persistent corpus luteum and endometritis (Fig. 1 to 3).

The rectal palpation showed that 56 out of 80 inseminated BX cows were failed to pregnant. Purposive sampling was done by selecting 18 BX cows, consisted of 6 BX cows with positive (+) NRR and rectal palpation; 6 BX cows with positive (+) NRR but negative (-) rectal palpation; and 6 BX cows with negative (-) NRR and rectal palpation, for USG examination. The estradiol 17-β levels of each cow were measured to understand the relationship of the hormone concentration to the status of pregnancy of the BX cows' ovary (Table 2).

Ultrasonography Examination

It can be seen that the highest 17β-estradiol concentration levels of the BX cows were 5,765 ng/ml while the lowest was 0.41 ng/ml, with each BX cow had varying 17β-estradiol concentration levels. The observed 17β-estradiol concentration levels in this study are higher than the finding by Domènech *et al*. (2011) in dairy cows, which showed 17β-estradiol concentration levels at 26.75±8.63 pg/ml or equals to 2,675 ng/ml; and Kajaysri (2006) which showed the 17β-estradiol concentration levels in Friesian crossbreed dairy cows were 2,786 ng/ml.

Ultrasonography Observation

Physiological observation of the BX cows' ovary by using ultrasonography showed that 6 cows (7%) had a follicular cyst, 2 cows (2.25%) had persistent corpus luteum and 1 cow (1.25%) had ovarian hypofunction.

Table 1: The pregnancy rate of Brahman Cross cows at the different insemination time interval

	Treatment		
Pregnancy Rate	P1	P2	Total
Successful pregnancy (%)	13 (32.5%)	11 (27.5%)	24 (30%)
Failed pregnancy (%)	27 (67.5%)	29 (72.5%)	56 (70%)
Total	40 (100%)	40 (100%)	80 (100%)

Note: P1 = BX cows inseminated at 0-2 h interval from estrous detection; P2 = BX cows inseminated at 8-10 h interval from estrous detection

Table 2: Status of pregnancy examinations of the Brahman Cross cows at different insemination time interval

	Non-Return Rate					
No	NRR_1	NRR_2	NRR_3	Rectal palpation	Estradiol 17-β concentration (ng/ml)	Result
1	+	+	+	-	1.13	Persistent corpus luteum was found
2	+	+	+	-	0.41	Ovarian hypofunction was found
3	+	+	+	-	2,618	Suspected early embryonic death
4	-	-	-	-	4,371	Follicular cyst was found
5	-	-	-	-	4,217	Follicular cyst was found
6	-	-	-	-	5,765	Follicular cyst was found
7	+	+	+	+	1,745	6-months pregnancy
8	+	+	+	+	1,611	6-months pregnancy
9	+	+	+	+	1,664	5-months pregnancy
10	+	+	+	-	2,517	Suspected early embryonic death
11	+	+	+	-	1.26	Persistent corpus luteum was found
12	+	+	+	-	2,575	Suspected early embryonic death
13	-	-	-	-	3,052	Follicular cyst was found
14	-	-	-	-	5,459	Follicular cyst was found
15	-	-	-	-	4,437	Follicular cyst was found
16	+	+	+	+	1,590	4-months pregnancy
17	+	+	+	+	1,312	5-months pregnancy
18	+	+	+	+	1,611	4-months pregnancy

Description: The non-return rate observations were performed at 18-days (NRR_1), 36-days (NRR_2) and 54-days (NRR_3) after insemination. The rectal palpation and ultrasonography examinations were performed 90-days after insemination.

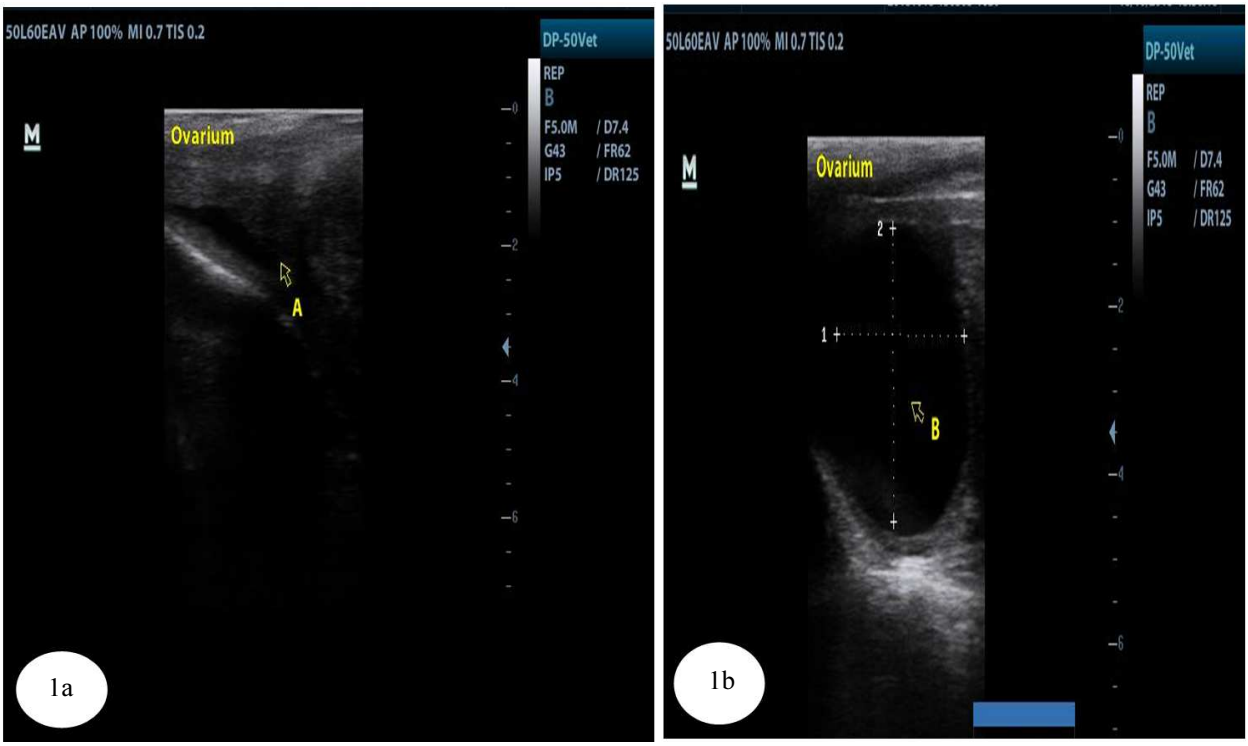

Fig. 1: (a) No follicle was found in the ovary and ovarian hypofunction has occurred; (b) Follicular cyst was found

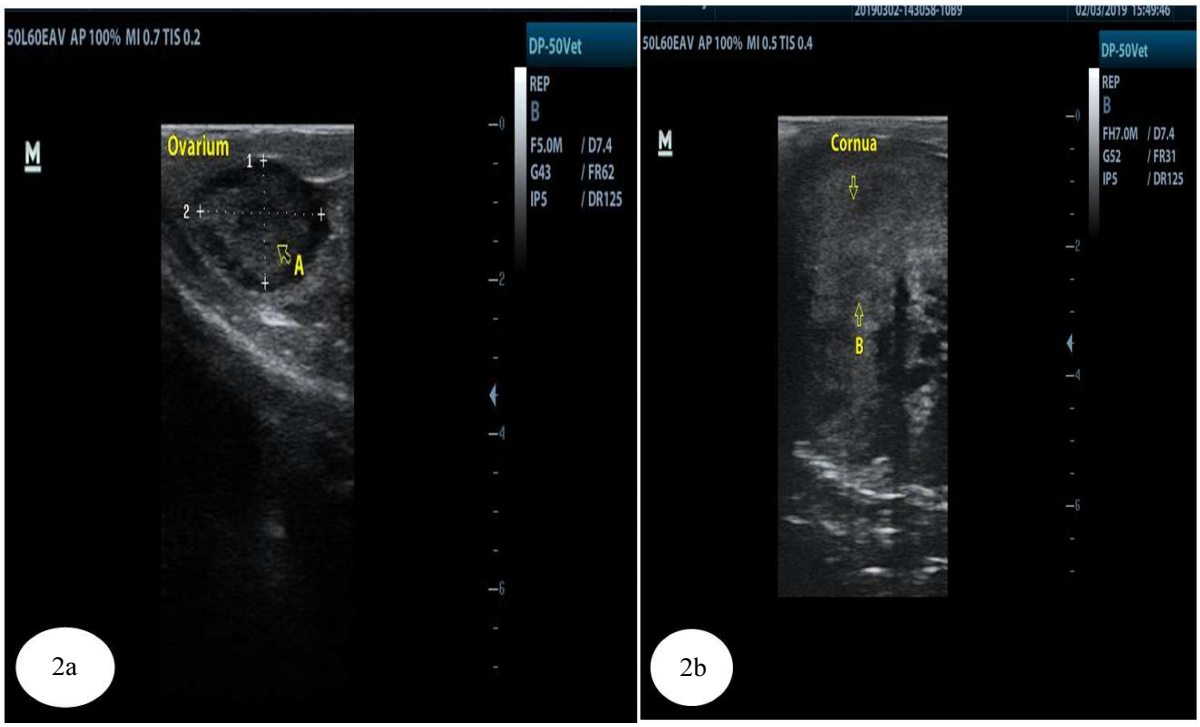

Fig. 2: (a) Persistent corpus luteum was found; (b) Endometritis is found in the uterus

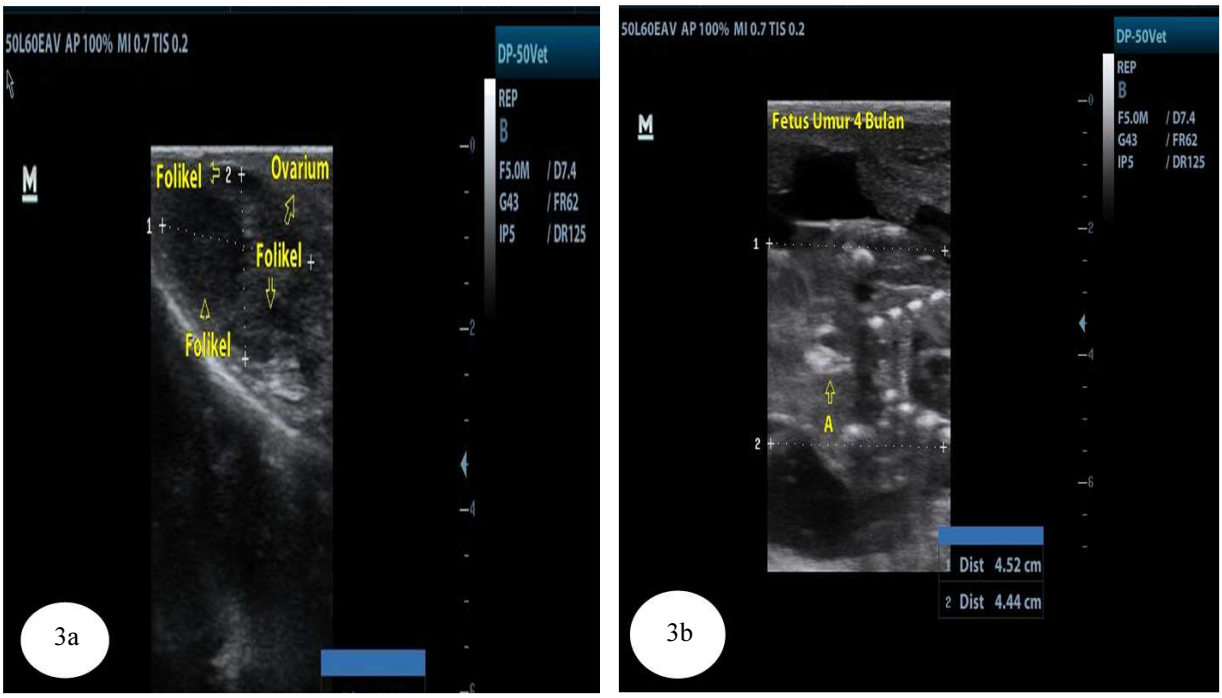

Fig. 3: (a) Normal ovary with the follicle; (b) 4-months fetus

In Fig. 1, it can be seen that the BX cows had ovarian hypofunction, characterized by the slippery ovarian surface during rectal palpation due to the undeveloped follicles and corpus luteum. It is allegedly caused by the feed deficiency of the BX cows which resulted in impaired FSH and LH secretion. Endometritis and persistent corpus luteum with 2.59 mm in length and 1.58 mm in width were also found in the BX cows in this

study (Fig. 2). The USG observation with normal ovary and 4-months fetus can be seen in Fig. 3.

Discussion

Pregnancy rate of the Brahman Cross cows

In this study, energy deficiency from the feed is known to contribute to the low rate of successful pregnancy, noting that it would affect the FSH and LH hormone secretion, thus disrupt ovulation, fertilization and embryonic development as well as causing ovarian hypofunction. Boland and Lonergan (2003) reported that nutrient deficiencies can reduce fertility, whereas Yekti *et al*. (2017) added that low feed quality and quantity will cause sub-fertilization, especially in exotic crossbreed cows. Zare *et al*. (2008) showed the impact of improved nutrition would initiate the hypothalamus to affect the anterior pituitary gland to release gonadotropins and growth hormones, thus affect ovulation followed by increased estradiol l7-β levels. Another known factor that contributes to the successful pregnancy is the proper heat detection of the inseminated cows. The heat occurrence on the acceptor is affected by gonadotropin and FSH hormone induction, which stimulates the development of primary and de Graff follicles, especially granulosa cells which, then produce the 17-β estradiol and LH hormone and stimulates ovulation (Hafez and Hafez, 2000).

Relationship between Estradiol 17-β Concentration and Ultrasonography Examination

A large follicular cyst was found in the BX cows' ovary (Fig. 1), which then caused nymphomanic condition (continuous heat) and affect the 17β-estradiol levels. This is in accordance with Nora *et al*. (2018), who showed that large follicles, as well as the occurrence of the follicular cyst, would result in high 17β-estradiol levels. The occurrence of a follicular cyst could be detected if the follicle is larger than 25 mm without any corpus luteum whether on both left or right ovary (Murayama *et al.*, 2015).

The blood sample collection in this study was carried out at 9 AM before the ultrasonography (USG examination. The time for blood collection is essential as the cows would have a varying physiological condition, thus affect its 17β-estradiol levels. Hafez and Hafez (2000) mentioned that during a oestrous cycle, reproductive hormones interact with each other and affect its physiological and behavioral changes of the cow. Hossner (2005) added that hormones are influenced by internal and external factors. The internal factors include the genetic and physiological conditions, while the external factors include nutrition intake and the environment condition.

The ultrasonography observation showed that 6 cows had follicular cysts, 2 cows had persistent corpus luteum and one cow had ovarian hypofunction. The follicular cyst would cause reproductive failure and mostly found with the diameters at more than 2.5 cm, attached in the ovary for 10 days or more, without any presence of corpus luteum. The follicular cyst is known to disrupt ovulation and decreased LH hormone production. Hermadi *et al*. (2011) explained that follicular cyst will occur due to the lack of LH hormone production, while the FSH hormone remained sufficient, thus stimulate the production of the anovulatory follicle and resulted in nymphomanic condition of the cow. Yekti *et al*. (2017) added that nymphomania in cow indicates the occurrence of follicular cysts.

Moreover, endometritis and persistent corpus luteum were also found in the BX cows in this study (Fig. 2). This condition was caused by whether infection or high progesterone levels suppress the FSH and LH secretion from the anterior pituitary gland. Research by Ghanem *et al*. (2015) has elucidate various bacterial infection to the occurrence of endometritis, while Sheldon *et al*. (2006) showed that high progesterone level in blood contributes to the persistent corpus luteum.

The ovarian hypofunction occurs due to the low FSH and LH hormones production and resulted in the failure of follicle production in the ovary. Hermadi *et al*. (2011) stated that the bad physiological condition, as well as long feed deficiency of the cow, would alter the ovarian hypofunction into ovarian atrophy. Moreover, the persistent corpus luteum would occur due to the failed luteolysis and the corpus luteum remained in the ovary in a long time despite the failed pregnancy. All form of the corpus luteum can produce the progesterone hormone, this explains that the BX cows with persistent corpus luteum in this study had high progesterone hormone levels in the blood. Boland and Lonergan (2003) mentioned that nutrition deficiency would reduce the cows' fertility and research by Zare *et al*. (2008) showed that, the improved nutritional intake would affect the anterior pituitary gland to produce higher gonadotropin and growth hormone, thus resulted in higher ovulation and 17β-estradiol levels. In addition, Feradis (2014) showed that nutrition deficiency would disrupt the gonadotropin synthesis in the pituitary gland.

Relationship between Non-Return Rate, Estradiol 17-β Concentration and Ultrasonography Examination

In this study, pregnancy examination was done by observing the NRR, rectal palpation and further confirmed by ultrasonography examination. The 17β-estradiol levels were also measured to understand its relationship to the status of pregnancy. The ultrasonography observation of the BX cows showed

various ovarian abnormalities, such as persistent corpus luteum, ovarian hypofunction and follicular cyst. Hafez and Hafez (2000) described that the ovarian hypofunction is caused by the failure of follicular cells to respond to the hormonal stimulation, changes in hormonal secretion, decreased stimulation in the ovarian hypothalamic-pituitary function which resulted in no ovarian activity after calving. The clinical symptoms of follicular cyst include nymphomania (continuous heat), anestrus (silent heat), sacroischiadicum ligament relaxation, vulvar edema and increased uterus size. Noakes *et al*. (2016) showed that follicular cyst with diameters at 1-3 cm can be felt when touched using rectal palpation. The occurrence of a follicular cyst would cause ovulation failure. Ovulation requires a series of processes at the cellular and subcellular levels involving hormones and factors in the form of complex biochemical compounds (Russell and Robker, 2007). The existence of metabolic infertility is closely related to malnutrition which will lead to the low reproductive performance of cows (Donaldson, 2014). In addition, Dibia *et al.* (2015) mentioned that another factor that needs to be further observed is the high cases of ovarian hypofunction in productive cows due to crossbreeding.

The occurrence of persistent corpus luteum (Fig. 2a) and endometritis (Fig. 2b) indicates that the BX cows suffer pyometra. Endometritis is caused by the failed PGF2α production in the endometrium, which then resulted in the failed luteolysis. Santoso *et al*. (2014) described that persistent corpus luteum often found in cows with reproductive disorders, indicated with continuous progesterone production and longer heat cycle. However, in some cases, cows could still get pregnant, but the fetus would still die to the failure involution (Sayuti *et al*., 2012; Cuneo *et al*., 2006). Siregar *et al*. (2012) described that small follicles (3-8) with a total of fewer than 10 follicles are categorized as dominant follicles, while small follicles with a total of more than 10 follicles were not. This study showed that the examination of dominant follicles is based on its morphology, the results of the study indicate that follicular growth is a dynamic process controlled by local and systematic processes (Fortune *et al*., 2004). Large follicles that appear during luteolysis will become dominant and subsequently ovulate in the follicular phase (Inskeep, 2004).

Pregnancy is a period from fertilization until giving birth (Frandson *et al*., 2009). The risk of failed pregnancy would be higher during early pregnancy, specifically at day-2 until day-42 after insemination. The early ultrasonography examination would produce more accurate failed pregnancy detection compared to rectal palpation (De-Vries, 2006). Moreover, feed also known to play important roles in the reproduction process. Research by Susilawati (2013) showed that feed would affect all cows' activities, starting from the cows' metabolism, growth, lactation and also reproductive activities. Therefore, special concern in nutritional intakes of the cows should be taken, especially in the proteins, carbohydrates, vitamins and mineral intakes, as the deficiency of the compounds would cause continuous reproductive disorders.

Conclusion

This study concludes that the 0-2 h artificial insemination interval after estrous detection of BX cows had a higher pregnancy rate (32.5%) compared to 8-10 h interval (27.5%). Moreover, the 17β-estradiol measurement showed that the highest 17β-estradiol levels of BX cows in this study were at 5,765 ng/ml, while the lowest at 0.41 ng/ml. The ultrasonography observation showed that 6 out of 80 BX cows (7%) had ovary abnormalities due to the occurrence of the follicular cyst, 2 cows (2.25%) had persistent corpus luteum and 1 cow (1.25%) had ovarian hypofunction.

Acknowledgement

We would like to thank the director and company staff of PT. Pasir Tengah (PT. Widodo Makmur Perkasa) for facilitating the research.

Funding Information

BUDI-DN Scholarship by the Indonesia Endowment Fund for Education (LPDP Ministry of Finance of the Republic of Indonesia) as the funders of this research.

Author's Contributions

Mohamad Ervandi and Trinil Susilawati: Designing and conducting the research and writing the manuscript.

Muhammad Nur Ihsan and Sri Wahjuningsih: Collaborating in field research, conducting laboratory analysis and interpreting the obtained data.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

Annashru, F.A., M.N. Ihsan, A.P.A. Yekti and T. Susilawati, 2017. The effect of differences time in artificial insemination toward successful Brahman Cross pregnancy. J. Ilmu-Ilmu Peternakan, 27: 17-23. DOI: 10.21776/ub.jiip.2017.027.03.03

Boland, M. and P. Lonergan, 2003. Effects of nutrition on fertility in dairy cows. Adv. Dairy Cattle Technol., 19: 155-160.

Cuneo, S.P., C.S. Card and E.J. Bicknell, 2006. Disease of beef catlle asociated with post-calving and breeding. The University of Wisconsin, Madison.

De-Vries, A., 2006. Economic value of pregnancy in dairy cattle. J. Dairy Sci., 89: 3876-3885. DOI: 10.3168/jds.S0022-0302(06)72430-4

Dibia, I.N., N.L. Dartini and N.M. Asrani, 2015. Cattle reproductive disorders in Lombok Island West Nusa Tenggara Province. Buletin Veteriner, 27: 1-10.

Domènech, A., S. Pich, A. Arís, C. Plasencia and A. Bach *et al*., 2011. Heat identification by 17β-estradiol and progesterone quantification in individual raw milk samples by enzyme immunoassay. Electronic J. Biotechnol., 14: 1-5. DOI: 10.2225/vol14-issue4-fulltext-6

Donaldson, R., 2014. Beef Cattle Breeding: Principles and Problems. 1st Edn., Rowell Press, Sydney, pp: 324.

Feradis, 2014. Cattle Reproduction. 1st Edn., Alfabeta, Bandung, ISBN-10: 9786028800082.

Fortune, J.E., G.M. Rivera and M.Y. Yang, 2004. Follicular development: The role of the follicular microenvironment in selection of the dominant follicle. Anim. Reproduct. Sci., 83: 109-126. DOI: 10.1016/j.anireprosci.2004.04.031

Frandson, R.D., W.L. Wilke and A.D. Fails, 2009. Anatomy and Physiology of Farm Animals. 7th Edn., Willey-Blackwell, New Jersey, ISBN-13: 9780813813943, pp: 436.

Fricke, P.M., 2005. Potential applications and pitfalls of ultrasound for managing reproduction in dairy Cattle. Vet. Clinics North Am. Food Anim. Pract., 21: 419-36. DOI: 10.1016/j.cvfa.2005.02.005

Ghanem, M.E., E. Tezuka, B. Devkota, Y. Izaike and T. Osawa, 2015. Persistence of uterine bacterial infection and its associations with endometritis and ovarian function in postpartum dairy cows. J. Reproduct. Dev., 61: 54-60. DOI: 10.1262/jrd.2014-051

Hafez, E.S.E. and B. Hafez, 2000. Follicullogenesis, Egg Maturation and Ovulation. In: Reproduction in Farm Animal, Hafez, B. and E.S.E. Hafez (Eds.), Lippincott Williams and Wilkins, Philadephia, PA, ISBN-13: 9780683305777, pp: 68-81.

Hermadi, A.H., M. Hariadi and Wurlina, 2011. Application of human menopose gonadotrophin (hmg) toward follicle growth in cows ovary hypofunction. Vet. Medika, 4: 239-245.

Hossner, K.L., 2005. Hormonal Regulation of Farm Animal Growth. 1st Edn., CABI Publishing, USA, ISBN-10: 0851990800, pp: 240.

Inskeep, E.K., 2004. Preovulatory, postovulatory and postmaternal recognition effects of concentrations of progesterone on embryonic survival in the cow. J. Anim. Sci., 82: E24-E39. DOI: 10.2527/2004.8213_supplE24x

Kajaysri, J., 2006. Comparison of estradiol concentrations and conception rate between cows with different estrus behaviors. Vet. J., 16: 15-22.

Kuswati and T. Susilawati, 2016. Industri Sapi Potong. 1st Edn., UB Press, Malang, ISBN-13: 9786024320973.

Murayama, C., E. Yamasaki, A. Miyamoto and T. Shimizu, 2015. Effect of Dedicator Of Cytokinesis 6 (DOCK6) on steroid production in theca cells of follicular cysts. Biochem. Biophys. Res. Commun., 462: 415-419. DOI: 10.1016/j.bbrc.2015.05.005

Noakes, D.E., T.J Parkinson and G.C.W. England, 2016. Veterinary Reproduction and Obstetrics. 9th Edn., Elsevier, London, ISBN-13: 9780702028878, pp: 103.

Nora, M., K. Rachid, G. Abdelmoumène, B.M. Hocine and A.M. Yassine, 2018. Characterization of ovarian follicular and cystic fluids in cows. Veterinaria, 67: 73-79.

Roelofs, J., F. Lopez Gatius, R.H. Hunter, F.J. Van-Eerdenburg and C. Hanzen, 2010. When is a cow in estrus? Clinical and practical aspects. Theriogenology, 7: 327-344. DOI: 10.1016/j.theriogenology.2010.02.016

Royal, M.D., A.O. Darwash, A.P.F. Flint, R. Webb and J.A. Woolliams *et al*., 2000. Declining fertility in dairy cattle: Changes in traditional and endocrine parameters of fertility. Anim. Sci., 70: 487-501. DOI: 10.1017/S1357729800051845

Russell, D.L. and R.L. Robker, 2007. Molecular mechanisms of ovulation: Co-ordination through the cumulus complex. Human Reproduct. Update, 13: 289-312. DOI: 10.1093/humupd/dml062

Saacke, R.G., 2008. Insemination factors related to timed AI in cattle. Theriogenology, 70: 479-484. DOI: 10.1016/j.theriogenology.2008.04.015

Santoso, S., A. Amrozi and H. Herdis, 2014. Gambaran ultrasonografi ovarium kambing Kacang yang disinkronisasi dengan hormon prostaglandin F2 alfa (PGF2α) dosis tunggal. J. Kedokteran Hewan, 8: 38-42.

Sayuti, A., J. Melia, Amrozi, Syarifuddin and Roslizawaty *et al*., 2012. Gambaran klinis sapi Piometra sebelum dan setelah terapi dengan antibiotik dan prostaglandin secara intra uteri. J. Kedoteran Hewan, 6: 99-101.

Sheldon, I.M., G.S. Lewis, S. LeBlanc and R.O. Gilbert, 2006. Defining postpartum uterine disease in cattle. Theriogenology, 65: 1516-1530.

Siregar, T.N., M.G. Eldora, J. Melia, B. Panjaitan and Yusmadi *et al*., 2012. Kehadiran folikel dominan pada saat inisiasi superovulasi menurunkan respons superovulasi sapi Aceh. J. Kedokteran Hewan, 6: 67-71.

Susilawati, T., 2011. Tingkat keberhasilan inseminasi buatan dengan kualitas desposisi semen yang berbeda pada sapi peranakan Ongole. J. Ternak Tropika, 12: 05-24.

Susilawati, T., 2013. Pedoman Inseminasi Buatan Pada Ternak. 1st Edn., UB Press, Malang, ISBN-13: 9786022034582.

Yekti, A.P.A., T. Susilawati, M.N. Ihsan and S. Wahjuningsih, 2017. Fisiologi Reproduksi Ternak: Dasar Manajemen Reproduksi. 1st Edn., UB Press, Malang, ISBN-13: 9786024322458.

Zare, S., A. Sadeghipanah, H.J., Barfourooshi and M.A. Emami-Mibody, 2008. Effects of equine Chorionic Gonadotropin (eCG) administration and flushing on reproductive performance in Nadooshan goats of Iran. African J. Biotechol., 7: 3373-3379.