Asian Journal of Conservation Biology, July 2023. Vol. 12 No. 1, pp. 3–9 AJCB: FP0205/76733
ISSN 2278-7666 ©TCRP Foundation 2023 https://doi.org/10.53562/ajcb.76733

Research Article

Determination of artificial insemination timing in Banteng based on follicle size and uterine enlargement

Dedi R. Setiadi[1*], M. Agil[1], Bongot Huaso Mulia[3], Ardyta Widianti[3], Dondin Sajuthi[2], Jansen Manansang[3], Yohana Tri Hastuti[3], Setyaningsih R. Liwa[3], R. Iis Arifiantini[1]

[1]*Division of Reproduction and Obstetric, School of Veterinary Medicine and Biomedical Science, IPB University, Indonesia*
[2]*Division of Veterinary Internal Medicine, School of Veterinary Medicine and Biomedical Science, IPB University, Indonesia*
[3]*Indonesia Safary Park, Cisarua, Bogor, Indonesia*

(Received: August 20, 2022; Revised: December 27, 2022; Accepted: January 03, 2023)

ABSTRACT

The Banteng (*Bos javanicus* d'Alton 1823) is an endemic species in Indonesia that is threatened with extinction and whose population continues to decline in the wild. The aim of this study is to determine the timing of artificial insemination (AI) in the Banteng based on follicle size and uterine enlargement. For this study, two female Banteng were used in Taman Safari Indonesia, Cisarua, Bogor. Habituation was performed to facilitate data collection. Optimal timing for AI is determined by examining the reproductive organs using Ultrasonography (USG) to detect changes in the uterus and ovaries and monitor clinical signs of estrus. Follicle size and uterine examination was performed on days -1, 0, and +1 for 3 estrus cycles. Insemination was performed 2-3 times during estrus until ovulation occurred. The results showed that the average follicle size of both Banteng was 1.09 ± 0.02 cm on day -1, 1.31 ± 0.02 cm on day 0, and 1.33 ± 0.03 cm on day +1. Artificial insemination was performed with a double dose of frozen semen when the Banteng were still rideable, and the follicle size ranged from 1.23-1.38 cm. Pregnancy was examined by ultrasound on day 21 after mating; both Banteng females were pregnant after AI. It can be concluded that AI technique can be used in captive Banteng breeding program by studying the development of follicles and uterine enlargement.

Key words: Banteng, timing of artificial insemination, frozen semen, follicular size and uterine enlargement

INTRODUCTION

The Banteng (*Bos javanicus* d'Alton 1823) is one of Indonesia's endangered native wildlife species, listed on the International Union for Conservation of Nature (IUCN) Red List. Breeding is needed to increase the Banteng population in situ and ex situ. The Banteng population can be increased ex situ through artificial insemination (AI), which is widely used in cattle. According to Waberski (2018), AI is a technology in livestock production that is essential for maintaining genetic diversity. Artificial insemination is also non-invasive, cost-effective, fundamental to other assisted reproductive technologies, and a promising biotechnology for domesticated and non-domesticated animals (Palmer *et al.,* 2012; Waberski, 2018).

Banteng, as wild animals, are certainly different than cattle in general. The success of artificial insemination in Banteng requires various data on female reproductive physiology, such as oestrus cycle length, duration of oestrus, and ovulation, to achieve optimal mating timing. Banteng oestrus has been little studied, mostly through simple behavioural observations (Setiadi *et al.,* 2020). Behaviour is one of the success factors in wildlife breeding programs, especially with natural mating. Banteng mating behaviour is almost the same as in cattle, e.g., mounting of each other (Setiadi *et al.,* 2020). A female in heat is receptive to males and is in normal physiological oestrus followed by ovulation (Palmer *et al.,* 2012); therefore, it can be used as a strategy to bring males and females together to ensure the reproductive process (Silva *et al.,* 2017). Observation of behaviour during oestrus is not sufficient for breeding with frozen semen. Studying follicle size and uterine enlargement during oestrus is important to determine the timing of ovulation. The aim of this study is therefore to determine the timing of artificial insemination in Banteng based on follicle size and uterine enlargement.

MATERIALS AND METHODS

The procedure for this research was approved by the IPB Animal Ethics Committee with certificate number 002/KEH/SKE/2020. This research was conducted from December 2019 to November 2020 in Taman Safari Indonesia (TSI) Cisarua, Bogor West Jawa, Indonesia (site coordinates: S 6°42′53.1136″ and E 106°57′ 3.24746).

Research Material

Two female Javanese banteng (Bos javanicus, d'Alton 1823) were used for this study at Taman Safari Indonesia (TSI) Cisarua Bogor. The banteng originated from Baluran, East Java, and were bred at Taman Safari Indonesia 2 (TSI 2) Prigen, Pasuruan, East Java. The two female Banteng named Andini and Uchi aged 13

[*]*Corresponding Author's E-mail: setiadidvm@gmail.com*

and 9 years respectively, from different lines, had sired offspring. Both bantengs are in clinically healthy condition and kept together in one area with separate enclosures. The banteng was housed in 4 x 5 m cages at night and released into the exhibit area in the morning until late afternoon. The animals were fed grass ad libitum in the exhibition area and up to 15-20 kg per head in the cage, while they received up to 2 kg of concentrate per head daily. Deworming is done every 6 months, drugs other than deworming are administered only under certain conditions.

Research methods

a. Banteng Habituation

Three months before the examination, the two Banteng's were habituated. This habituation served to train the Banteng to go into the service box and to behave calmly during the examination. The habituation is performed gradually. In the first phase, the animals were let into the cage, where they remained for 5-10 minutes while they were given additional food, and then released into the exhibition area. This phase is done every day for a month. In the second phase, the animals are touched on the outside of the reproductive organs for 5-10 minutes, fed and then released. In the third phase, the animals are palpated rectally for 1-2 minutes and then released again while they continue to be fed. In the third phase of the habituation process, the rectal palpation is done with increased time intensity. Habituation is complete when the banteng has been quiet in the cage and can be palpated for 15 minutes, as the assessment of follicular development takes 10-15 minutes.

b. Assessment of uterine changes and follicular development in oestrus banteng

Evaluation of changes in the uterus and development of follicle size was performed after the appearance of the first oestrus symptoms (D-1) before the appearance of mounting behaviour (D0). The first observed oestrus symptom was the appearance of interest by the target female in riding another female, but the ridden female avoided. The change in symptoms after oestrus (D+1) is indicated by the behaviour of mutual attraction, but no female is inactive during mounting. The study of follicle size and uterine changes was performed over three oestrous cycles. Follicles were measured by ultrasound (USG Exago®ECM Noveko International Inc.) using a linear transducer with a frequency of 5 MHz. Ovarian follicle diameter was measured using USG callipers (Keskin *et al.,* 2016). The size of the uterus is determined by measuring the length and width of the uterus image appearing on the monitor. The measurements of follicular size and uterine enlargement were performed at D-1, D0 and D+1.

c. Artificial Insemination and Pregnancy diagnosis

Determination of the optimal time of mating (sperm deposition) occurs when the size of the dominant follicle is 1.2 - 1.4 cm (Hansen, 2020) and is also associated with changes in the uterus. Insemination is no longer performed when the dominant follicle has ovulated (identified as corpus rubrum). Artificial insemination was performed with frozen Banteng semen from TSI. The insemination dose per straw was $25x10^6$ sperm, with post-thaw motility (PTM) of 30% (7.5x106 motile sperm).

Insemination was performed three times during oestrus until ovulation occurred. Insemination equipment was used as for AI in cattle. Pregnancy diagnosis was made 20 days after insemination with transrectal ultrasound to determine the presence of the embryo in the amniotic sac and re-examination with ultrasound and rectal palpation on day 60 to ensure continuation of pregnancy.

Statistical analysis

Statistical analysis of the obtained follicle size data was performed using SPSS (version 21.0). Analyses were delivered in terms of the mean and standard error of the mean (SEM) for normally distributed variables.

RESULTS

It takes three months to acclimatise the Banteng until it is easy to handle and feels safe, calm and comfortable in a cage (Figure 1).

Changes in the uterus and follicle development during oestrus in Banteng

The uterus of banteng one day before oestrus (H-1), during oestrus (H0), and on the day after oestrus (H+1) looked different according to the results of ultrasonography (Figure 2). Differences were noted in the increase in uterine size and accumulation of mucus in the uterus (Miciakova *et al.*, 2018).

Figure 1. The habituation process of banteng in a service crate before enters (a), banteng inside the service crate (b), keeper giving supplementary feed (c1, c2).

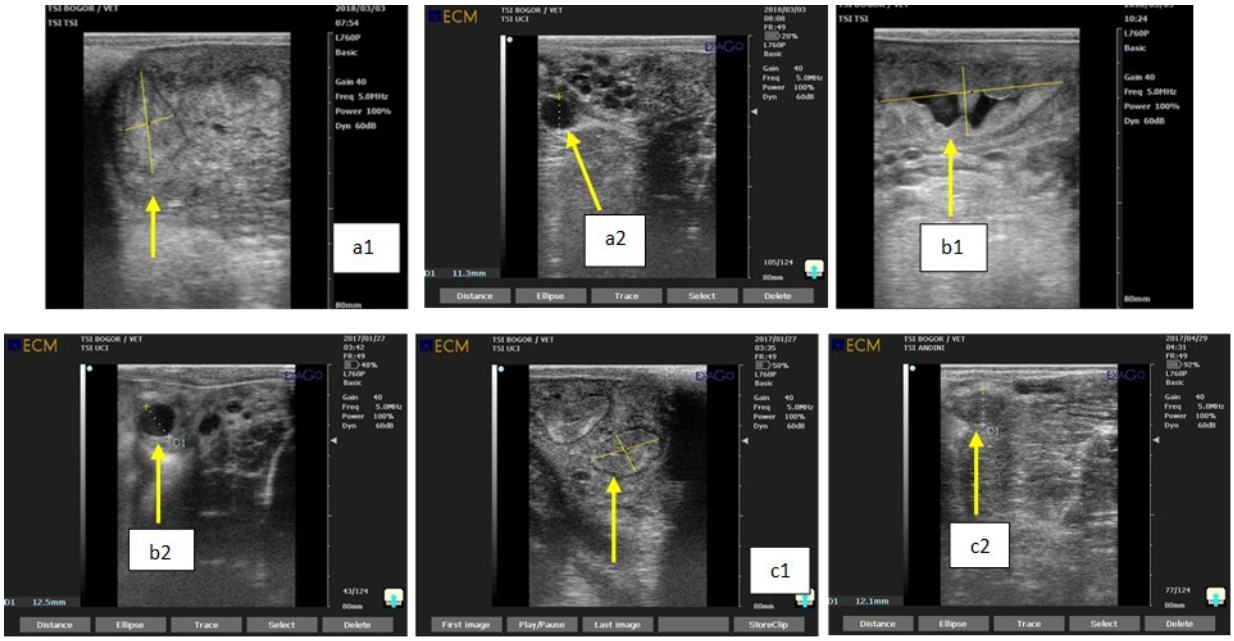

Figure 2. Uterine and ovarian changes by ultrasound on day-1 (a1) uterus (a2) follicles, on day 0 (b1) uterus (b2) follicles and day +1 (c1) uterus (c2) ovulation follicles. Images were taken using a 5 MHz frequency probe.

Figure 3. Changes in follicle size at D-1(a), D0 (b) and D+1 (c)

The uterus begins to tighten at D-1, its tension increases during massage. The accumulation of mucus in the uterus is still low. In addition, the condition of the uterus at D0 increases the tension and the accumulation of mucus in the uterus. Follicle size increases from D-1 to D0. From D0 to D+1, follicle size increases slightly, but no differences were found from D-0 to D+1. The maximum follicle size of Banteng in this study was 1.33 cm.

Artificial Insemination in Banteng

Artificial insemination is performed on day 0 until ovulation, when the female is receptive to others and the follicle has reached a size of 1.23 - 1.38 cm, and when the uterus has reached its maximum tension and mucus fluid is still accumulating (Figure 3).

DISCUSSION

It takes 3 to 6 months to acclimate the banteng until they are calm in the cage and can be rectally palpated (Setiadi *et al.,* 2018a). Habituation occurs more rapidly after a series of stimulus repetitions and spontaneous recovery (Blumstein, 2016). Feeding during habituation is effective for many animals (Abramson and Kieson 2016; Bell and Peeke, 2012). According to Claxton (2011), training also reduced the occurrence of group aggression during feeding time, which was considered to indicate stress reduction. According to Hosey (2013), a bond between humans and animals is considered established when the animal and human have interacted to some degree and can predict each other's behaviour.

According to Ahmadi *et al*. (2019), the uterus thickens and mucus accumulates during oestrus. Changes in uterine thickness, the presence of oedema, and the accumulation of intrauterine and intravaginal fluid during oestrus can be detected by ultrasound (Sugiura *et al.,* 2018). In addition, Perry *et al.* (2020) note that the composition and differentiation status of the endometrium change during the oestrous cycle. Ovarian status can be assessed by rectal palpation (Long *et al.,* 2021), but this technique is imprecise. Baez *et al.* (2015) note that several ultrasound features of the ovaries and uterus, such as CL diameter and its echo texture, follicle diameter, and uterine shape and echo texture, can be used to assess the oestrous cycle in cattle. Mihandoost *et al.* (2018) state that fluid accumulation in the uterine lumen can be visualized. Uterine tension at D+1 has decreased and oestrus mucus is lower.

The mean uterine length and width of the Andini and Uchi Banteng from this study were 2.08 ± 0.32 cm and 1.46 ± 0.24 cm, respectively. On day 1, it was 2.08 ± 0.32 cm and 1.46 ± 0.24 cm and increased to 4.49 ± 0.50 cm and 1.61 ± 0.11 cm, respectively on day 0. On day +1, uterine size decreased again by 1.88 ± 0.42 and 1.06 ± 0.22 cm, respectively (Table 1). The increase in endometrial thickness occurs approximately 3-4 days before ovulation and decreases 1-3 days after ovulation (Jimenez-Krassel *et al.,* 2009).

Table 1. Changes in uterine size of Andini and Uchi Banteng on D-1, D0 and D+1 for three oestrus cycles

Banteng/cycles	D-1		D0		D+1	
Andini	Length	Width	Length	Width	Length	Width
1st Oestrous cycles	1.69	1.36	5.19	1.54	1.54	1.04
2nd Oestrous cycles	2.02	1.27	4.29	1.62	1.21	0.85
3rd Oestrous cycles	1.79	1.24	4.57	1.54	2.06	0.98
Uchi						
1st Oestrous cycles	2.11	1.8	4.16	1.55	2.1	1.28
2nd Oestrous cycles	2.58	1.38	4.89	1.83	2.03	0.82
3rd Oestrous cycles	2.26	1.72	3.81	1.59	2.35	1.37
Mean± SD	2.08±0.32	1.46±0.24	4.49±0.50	1.61±0.11	1.88±0.42	1.06±0.22

Table 2. Follicular growth of Banteng during oestrous in three oestrous cycles (Mean±SE)

Banteng	Oestrous cycle	Day	Follicle size (cm)	Day	Follicle size (cm)	Day	Follicle size (cm)
Andini	1	-1	1.07	0	1.23	+1	1.26
	2	-1	1.15	0	1.36	+1	1.36
	3	-1	1.09	0	1.31	+1	1.31
Uchi	1	-1	1.09	0	1.25	+1	1.25
	2	-1	1.13	0	1.38	+1	1.38
	3	-1	1.03	0	1.3	+1	1.4
Mean			1.09±0.02a		1.31±0.02b		1.33±0.03b

Different letters following numbers in the same row indicate differences ($p<0.05$

As shown in Table 1, the uterus of both Andini and Uchi Banteng was enlarged during oestrus and had a length of 4.44 cm to 4.54 cm and a width of 1.51 cm to 1.72 cm. The enlargement of the uterus is closely related to the condition of oedema, thickening of the uterus, and accumulation of mucus in the uterus. The uterus is larger in D0 than in D-1 or D+1 (Ahmadi *et al.*, 2019). The uterus is thicker in oestrus than in dioestrus. Uterine thickness increases during normal luteolytic and reaches its maximum thickness before ovulation (Sugiura *et al.,* 2018).

Examination of the ovaries of both banteng on days -1 and +1 for three oestrous cycles showed significant growth in follicle size as shown in (Table 2). The size of the follicle in this study is almost equal to the size previously determined by Setiadi et al. (2018b) as 1.2-1.4 cm. According to Pfeifer *et al.* (2012), there are differences in follicle size between beef and dairy cows. Follicle size in cattle is 1.3-1.5 cm, while in dairy cows it is 1.4-1.5 cm (Monroy *et al.*, 2018). Another report in Aceh cattle, an Indonesian local breed, showed 1.23-1.26 cm (Armansyah *et al.,* 2017). In general, the diameter of the dominant follicle during the oestrus cycle in cattle ranges from 1.0 to 2.0 cm (Lemma, 2013).

The results of follicle size examination were consistent with clinical signs; in D0, clear mucus was seen (Figure 4) and follicles were quiescent when mounted by others (Figure 5). No true clinical signs were observed in D-1 and D+1.

According to Orihuela (2000) and Palmer *et al.* (2012), standing still when mounted is the most reliable oestrus sign and the best indicator that the female is ready to mate. This fact is also the main indicator of oestrus in Bali cattle (Kune *et al.*, 2019). The intensity of oestrus is the most important factor for the accuracy of oestrus detection and differences between animals (Siregar *et al.,* 2015). In addition, Keskin *et al.* (2016) mentioned that the oestrus intensity is high when the animal is sitting still. In addition, the method of heat detection at rest was cited as the only criterion for the cow's heat (Palmer *et al.,* 2012).

All banteng were pregnant after three artificial inseminations. Artificial insemination was repeated because the frozen semen had low sperm motility. After thawing, motility is below 30% even in a straw with $25x10^6$ sperm. To ensure fertilization, double the dose is administered. In addition, the timing of ovulation is unknown. In this study, artificial insemination was performed when the diameter of the follicles was 1.3 cm. In Swiss Brown Swiss, German Simmental and Holstein cattle, artificial insemination was also performed when the follicle diameter was 1.20 - 2.25 cm (Luttgenau *et al.,* 2015). In another study in Hereford and Charolais cattle, insemination was performed when the follicle size was 1.3 - 1.5 cm (Pfeifer *et al.*, 2012). In addition, in Swedish FH and Red cattle (Keskin *et al.,* 2016), insemination is performed when the mean of follicle size is 1.45 cm and 1.75 cm.

Pregnancy examination by rectal palpation on the 60th day after AI to confirm the ultrasound result on day 18th and ensure that the banteng is pregnant. Szenci (2021) also reported confirmation of ultrasound results by rectal palpation 2 to 3 months after AI. This method was used because of the frequency of premature embryo death. In addition, accurate early pregnancy screening can be performed with ultrasound at day 20 to day 30 (Adams and Singh 2011; Abdullah *et al.* 2014), Scully *et al.* 2014; Bekele *et al.* 2016). We can even examine heart rate on day 21 post-mating (Balhara et al. 2013).

This study is the first report on the success of AI of banteng with frozen semen. The three inseminations in this study are impractical and time consuming. If the exact time of ovulation is known and good quality frozen semen is used, insemination can be performed only once in the future, and it is expected that the same pregnancy rate as this artificial insemination will be achieved.

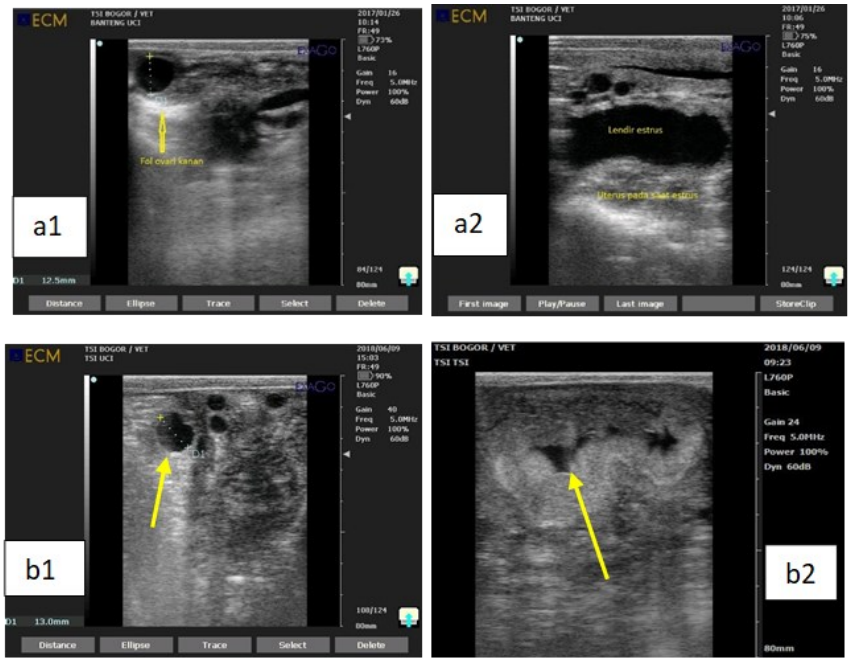

Figure 4. Ultrasound view of the follicle and uterus at D-0 (a1, a2) and D+1 (b1, b2,) follicle size 1.23 cm (a1) and accumulation of mucus in the uterus (a2), size 1.38 cm follicle (b1) and reduced of mucus accumulation in the uterus (b2).

Figure 5. Oestrous behaviour of female Banteng in the exhibition area

CONCLUSION

The time of insemination in banteng is when the female is still being ridden and the follicles reach a size of 1.23-1.38 cm, the uterus reaches maximum tension and estruses mucus accumulates. The use of ultrasound to determine ovarian status and diagnose pregnancy can increase the success of artificial insemination programs in banteng. Artificial insemination techniques can be used for captive (*ex situ*) banteng breeding programs.

CONFLICT OF INTEREST

The researcher declares that there is no financial, personal or other conflict of interest with other people or organizations related to the material discussed in this article

ACKNOWLEDGEMENT

We thank Taman Safari Indonesia (TSI) Cisarua Bogor for funding and facilitating the research, the School of Veterinary Medicine and Biomedical Sciences IPB University - TSI collaborative team and especially the TSI veterinarians and Banteng keepers who always provided technical support during the research.

REFERENCES

Abramson, C. I. and Kieson, E. 2016. Conditioning methods for animals in agriculture: a review. *Ciencia Animal Brasileira.* 17(3): 359-375. doi: 10.1590/1089-6891v17i341981.

Abdullah, M., Mohanty, T. K., Kumaresan, A., Mohanty, A. K., Madkar, A.R. Baithalu, R. K. and Bhakat, M. 2014. Early pregnancy diagnosis in dairy cattle: economic importance and accuracy of ultrasonography. *Advances in Animal and Veterinary Science.* 2(8): 464-467. doi http://dx.doi.org/10.14737/journal.aavs/2014/2.8.464.467.

Adams, G. P. and Singh, J. 2011. Bovine Bodyworks: Ultrasound Imaging Of Reproductive Events in Cows. *WCDS Advances in Dairy Technology*. 23:239-254.

Ahmadi, M. R., Mogheisehi, A., Mihandoost, B. and Lari, M. A. 2019. Ultrasonic characteristics of the uterus and ovaries during estrus, and their relationship with pregnancy rate in dairy cows. *Veterinarski Arhiv*. 89(3):279-294. doi: 10.24099/vet.arhiv.0253.

Armansyah, T., Siregar, T. N., Aliza, D., Melia, J., Meutia, N., Panjaitan, B., Hafizuddin, Adam, M. and Abrar, M. 2017. Aceh cattle follicle dynamic under environmental heat stress. *Jurnal Kedokteran Hewan*. 11(3):86-90. doi: https://doi.org/10.21157/j.ked.hewan.v11i3.6377.

Balhara, A. K., Gupta, M., Singh, S., Mohanty, A. K. and Singh, I. 2013. Early Pregnancy Diagnosis in Bovines: Current Status and Future Directions: Review Article. *The Sciece W J.* 5:1-10. http://dx.doi.org/10.1155/2013/958540.

Baez, G. M., Barletta, R. V., Guenther, J. N., Gaska, J. M. and Wiltbank. M. C. 2015. Effect of uterine size on fertility of lactating dairy cows. *Theriogenology*. doi: 10.1016/ j.theriogenology.2015.04.022.

Bekele, N., Addis, M., Abdela, N. and Ahmed, W. M. 2016. Pregnancy diagnosis in cattle for fertility management : A Review. *Global Veterinary*. 16 (4):355-364. doi: 10.5829/ idosi.gv.2016.16.04.103136.

Bell, A. M. and Peeke, H. V. S. 2012. Individual variation in habituation: behaviour over time toward different stimuli in threespine sticklebacks (Gasterosteus aculeatus). *Behaviour.* 149(13-14):1339–1365. doi:10.1163/1568539X-00003019.

Blumstein, D. T. 2016. Habituation and sensitization: new thoughts about old ideas. *Animal Behaviour*. 120. 255-262.

Claxton, A. M. 2011 The potential of the human–animal relationship as an environmental enrichment for the welfare of zoo-housed animals. Applied Animal. Behaviour Science. 133(3):1–10. doi:10.1016/j.applanim.2011.03.002.

Hosey, G. 2013. Hediger Revisited: How Do Zoo Animals See Us?. *Journal of Applied Animal Welfare Science*. 16(4):338–359. doi: 10.1080/10888705.2013.827916.

Hansen, P. J. 2020. The incompletely fulfilled promise of embryo transfer in cattle—why aren't pregnancy rates greater and what can we do about it?. *Journal of Animal Sciece*. 98(11):1–20. doi:10.1093/jas/skaa288.

Keskin, A., Mecitoglu, G., Bilen, E., Güner, B., Orman, A., Okut, H. and Gumen, A. 2016. The effect of ovulatory follicle size at the time of insemination on pregnancy rate in lactating dairy cows. *Turkish Journal of Veterinary and Animal Sciences.* 40(1):68–74. doi:10.3906/vet-1506-59.

Kune, P., Widyastuti, R. and Saili, T.2018. Tampilan kesuburan sapi bali induk yang dikawinkan langsung dengan pejantan dan inseminasi buatan ketika estrus hasil sinkronisasi menggunakan PGF2α. *Jurnal dan Ilmu Teknologi Perikanan Tangkap*. 6 (2):275-280. doi: http://dx.doi.org/10.33772/ jitro.v6i2.7142.

Jimenez-Krassel, F., Folger, J. K., Ireland, J. L. H., Smith, G. W., Hou, X., Davis, J. S., Lonergan, P., Evans, A. C. O. and Ireland, J. J. 2009. Evidence That high variation in ovarian reserves of healthy young adults has a ngative impact on the corpus luteum and endometrium during estrous cycles in cattle. *Biology of Reproduction*. 80 (1):1272–81. doi: 10.1095/ biolreprod.108.075093.

Lemma, A. 2013. The Role of Trans-Rectal Ultrasonography in Artificial Insemination Program. Success in Artificial Insemination-Quality of Semen and Diagnostics Employed. *INTECH*. 1(8):141-152. doi:10.5772/52721.

Luttgenau, J., Mang, H., Borel, N.. Bruckmaier, R. and Bollwein, H. 2015. Ultrasonographic examination reduces the percentage of unsuccessful inseminations in dairy cows. *Theriogenology*. 10. doi: 10.1016/j.theriogenology.2015.10.004.

Longa, S. T., Gioib, P. V. and Suongc, N. T. 2021. Some Factors Associated with Ovarian Disorders of Dairy Cattle in Northern Vietnam. *Tropical Animal Science Journal.* 44(2):240-247. doi: https://doi.org/10.5398/tasj.2021.44.2.240

Miciakova, M., Strapak, P., Szencziova, I, Strapakova, E. and Hanusovsky. O. 2018. Several methods of estrus detection in cattle dams: a review. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis.* 66(2):619-625. doi: 10.11118/actaun201866020619.

Mihandoost, B., Mogheiseh, A., Nazifi, S., Ahmadi, M. R. and Ansari-Lari, M. 2018. Association between hormonal and structural changes of the reproductive tract in estrus dairy cows. *Comparative Clinical Pathology*. 4. https:// doi.org/10.1007/s00580-018-2716-3.

Monroy, G. M., Jiménez, E. C. and Hernández, V. A. 2018. Follicular and luteal morphometry, progesterone concentration and early gestation in Holstein cows (Bos Taurus) at high altitude in the tropics (Colombia). *Revista MVZ Cordoba*. 23(2). 6681-6695. doi: doi.org/10.21897/ rmvz.1342.

Orihuela, A. 2000. Some factors affecting the behavioural manifestation of oestrus in cattle: a review. *Applied Animal Behavior Science*. 70 (4):1-16. doi: 10.1016/S0168-1591(00)00139-8.

Palmer, M. A., Olmosc, G., Boylec, L. A. and Meec, J. F. 2012. A comparison of the estrous behavior of Holstein-Friesian cows when cubicle-housed and at pasture. *Theriogenology.* 77(8):382–388.

Perry, G. A., Cushman, R. A., Perry, B. L., Schiefelbein, A. K., Northrop, E. J., Rich, J. J. J. and Perkins, S. D. 2020. Role of preovulatory concentrations of estradiol on timing of conception and regulation of the uterine environment in beef cattle. *Systems Biology in Reproductive Medicine*. 66(1):12-25. doi: 10.1080/19396368.2019.1695979.

Pfeifer, L. F. M., Souza Leal, S. C. B., Schneider, A., Schmitt, E. and Corrêa, M. N. 2012. Effect of the ovulatory follicle diameter and progesterone concentration on the pregnancy rate of fixed-time inseminated lactating beef cows. *Revista Brasileira de Zootecnia.* 41(4):1004-1008.

Scully, S., Butler, S. T., Kelly, A. K., Evans, A. C. O., Lonergan, P.and Crowe, M. A. 2014. Early pregnancy diagnosis on days 18 to 21 post insemination using high-resolution imaging in lactating dairy cows. *Journal Dairy Science.* 97(6):3542–3557. http://dx.doi.org/ 10.3168/jds.2013-7518.

Setiadi, D. R., Agil, M., Arifiantini, I., Yusuf, T. L., Sajuthi, D., Noordin, M., Hastuti, Y. T., and Liwa, S. R. 2018a. Habituation process for assesseing ovarian cycle using ultrasonography (usg) to establish artificial insemination program in banteng (*Bos javanicus*). Poster Presentation. Proc. of the 11th International Conference of Asian Society of Conservation Medicine. Bali Oct 28-30, 2018. 98

Setiadi, D. R., Agil, M., Arifiantini, I., Yusuf, T. L., Noordin, M., Hastuti, Y. T., Liwa, S. R., Manansang, J., and Sajuthi, D. 2018b. Assessment of estrous cycle using ultrasound to determine time

of insemination in banteng (*Bos javanicus*, d'Alton 1823). Oral Presentation. Proc. of the 20th fava congress & The 15th KIVNAS PDHI. Bali Nov 1-3, 2018. 56-60 .

Setiadi, D. R., Agil, M., Arifiantini, I., Sajuthi, D., Manansang, J., Hastuti, Y. T. and Liwa, S. R. 2020. Vaginal cytology characteristics during estrous cycle and clinical sign during estrus in Banteng (*Bos javanicus* d'Alton 1823). *Acta Veteranaria Indonesiana.* 8(3):40-47.

Silva, A. R., Moreira, N., Pereira, A. F., Peixoto, G. C. X., Maia, K. M., Campos, L. B. and Borges, A. A. 2017. Estrus Cycle Monitoring in Wild Mammals: Challenges and Perspectives. *Theriogenology.* 21-45. http://dx.doi.org/10.5772/intechopen.69444.

Siregar, T. N., Hamdan, H., Riady, G., Panjaitan, B., Aliza, D., Pratiwi, E. F., Darianto, T. and Husnurrizal. 2015. Efficacy of Two Estrus Synchronization Methods in Indonesian Aceh Cattle. *International Journal of Veterinary Science*. 4(2): 87-91.

Sugiura, T., Akiyoshi, S., Inoue, F., Yanagawa, Y., Moriyoshi, M., Tajima, M. and Katagiri, S. 2018. Relationship between bovine endometrial thickness and plasma progesterone and estradiol concentrations in natural and induced estrus. *Journal of Reproduction and Development*. 64 (2). 135-143.

Szenci, O. 2021. Recent Possibilities for the Diagnosis of Early Pregnancy and Embryonic Mortality in Dairy Cows. Animals. 11(1666):1-18. https://doi.org/10.3390/ani11061666.

Waberski, D. 2018. Artificial Insemination in Domestic and Wild Animal Species. *Animal Biotechnology*. 1. 37–64. doi:10.1007/978-3-319-92327-7_3.