



Effect of Artificial Insemination Timing on Conception Rate in Lactating Holstein-Friesian Cows

Habib Asshidiq Syah¹, Aulia Puspita Anugra Yekti¹, Putri Utami¹, Nurul Isnaini¹, and Trinil Susilawati*¹

Department of Animal Reproduction and Breeding, Universitas Brawijaya, Malang 65145, East Java, Indonesia

*Corresponding author's Email: tsusilawati@ub.ac.id

ABSTRACT

Successful pregnancy in dairy cows requires accurate timing of artificial insemination (AI). Artificial insemination conducted in the morning, midday, and afternoon exposes cows to different environmental temperatures with high ambient temperatures can potentially reduce the viability of spermatozoa, thus disrupting the fertilization process and increasing the possibility risk of pregnancy failure. The purpose of the present study was to ascertain and determine how the effect of various AI timings (during the morning, midday, and afternoon) affected the pregnancy success of the pregnancies in Holstein-Friesian cows. The purposive sampling was used to select a total of 191 Holstein-Friesian cows based on the following specific criteria; cows showing symptoms of estrus, having healthy reproductive organs, having one parturition at least once, aged 2-6 years old, having body condition score of 2.5-3.5 (on a 1-5 scale). The cows were divided into 3 three treatment groups including T1 cows inseminated from 06.00 am to 10.59 am (n=38 cows), T2 cows inseminated from 11.00 am to 03.59 pm (n=82), and T3 cows inseminated from 04.00 pm to 08.59 pm (n=71). The non-return rate was monitored at 19-22 days post-insemination (NRR-1) and 39-42 days post-insemination (NRR-2) was monitored, while the conception rate (CR) was assessed on day 60 post-insemination. Artificial insemination was performed 8 hours after estrus. NRR-1 values for T1, T2, and T3 were 82%, 80%, and 89%, respectively, The NRR-2 values were 71% in T1, 66% in T2, and 79% in T3. The CR for T1, T2, and T3 were 50%, 48%, and 54%, respectively. Although AI timing did not yield conception rates based on AI timing did not show a significant difference in conception rates, artificial insemination performed in the morning insemination is recommended due to its higher likelihood of successful pregnancy compared to other times.

Keywords: Artificial insemination, Conception rate, Dairy cow, Insemination timing, Lactating dairy cows, Non-return rate

INTRODUCTION

In dairy cows, achieving successful pregnancy requires artificial insemination (AI) at the right time. Accurate timing is crucial since AI conducted too early or too late can decrease the probability of pregnancy. Early AI decreases the chance of fertilization (Fernandez-Novo et al., 2020). Spermatozoa will age, and if ovulation occurs during this period, spermatozoa will not be able to fertilize the egg (Hawk, 1987). Spermatozoa that spend too long in the female reproductive tract will experience decreased motility due to excessive capacitation (Aitken et al., 2015). Capacitation is a physiological change that enables spermatozoa to fertilize the egg, but if this process is prolonged without fertilization, spermatozoa may lose their energy and ability to move actively (Mahdavinezhad et al., 2021). Sperm aging plays a critical role in reducing fertility in mammals, as it leads to alterations in membrane integrity and an increase in lipid peroxidation, ultimately decreasing the proportion of viable sperm capable of successful fertilization (Am-in et al., 2011; Donnellan et al., 2022). On the contrary, late AI can lead to the aging of the egg and disrupt the fertilization process (Hunter and Greve, 1997).

According to Roelofs et al. (2006), AI conducted 12-24 hours before ovulation has a high success rate. However, the absence of a definite sign of ovulation is an obstacle for small-scale farmers in determining the optimal AI time. Therefore, AI based on the onset of estrus is an option that can be used by farmers in deciding on an appropriate AI time. The optimal AI time is 8 hours after the onset of estrus (Marques et al., 2024). Therefore, AI conducted at 8 hours after estrus could lead to the possibility of AI in the morning, midday, and afternoon. Artificial insemination conducted during the midday is potentially detrimental to pregnancy success because daytime temperatures are higher than morning and afternoon temperatures (Hamid et al., 2018; Szenci et al., 2018). Elevated temperatures negatively impact sperm quality, potentially reducing pregnancy success (Hansen et al., 2001).

Properly timed AI is essential to maximize the probability of pregnancy on the first insemination attempt. Artificial insemination failure leads to various disadvantages due to longer feed costs, extended time required for conception, and a decrease in the number of offspring that can be born during the lifetime (Tadesse et al., 2022). Therefore, the objective of this study was to investigate whether the morning, midday, and afternoon AI times influence the success of conceptions among Holstein-Friesian cows.

ORIGINAL ARTICLE
Received: September 26, 2024
Revised: October 23, 2024
Accepted: November 27, 2024
Published: December 25, 2024

MATERIALS AND METHODS

Ethical approval

Ethical approval was given by the ethics committee of the Institute of Biosciences, Universitas Brawijaya, Malang, East Java, Indonesia, in compliance with ethical guidelines regarding responsible behavior in the use of Holstein-Friesian Holstein cows in experimental animal research (Ethical clearance number: 47/EC/KEPK/02/2024).

Animals and treatment groups

The cows used in this study consisted of 191 Friesian Holstein-Friesian cows owned by members of Sinau Andandani Ekonomi (SAE) Cooperative Pujon, a private dairy farm cooperative. In this study, the cows were divided into 3 three treatment groups: Treatment 1 (T1) with 38 cows inseminated from 06.00 am to 10.59 am, treatment 2 (T2) with 82 cows were inseminated from 11.00 am to 03.59 pm, and treatment 3 (T3) with 71 cows were inseminated from 04.00 pm to 08.59 pm. The difference in the number of cows per treatment is due to variations in the availability of cows in each period. The cows included in this study met the following criteria first, they had given birth at least once. Cows that have given birth indicate that the cow had normal reproductive organs capable of pregnancy and giving birth, as evidenced by the cow being able to get pregnant and give birth normally (Diskin, 2014). Second, cows had normal reproductive organs, as evidenced by rectal palpation before artificial insemination (AI). Third, cows were between 2 and 6 years old. According to the report of Susilawati (2014) cows older than six years tend to exhibit lower fertility rates compared to their younger counterpart. Fourth, cows had a Body Condition Score (BCS) of 2.5 to 3.25 (on a 1-5 scale). According to Yamada *et al.* (2003), BCS of 2.75-3.25 is associated with better AI outcomes. Fifth, the cows exhibited clear signs of estrus.

Artificial insemination

Artificial insemination was carried out by experienced, nationally certified local inseminators who are experienced and nationally certified. To minimize stress during the AI process, cows were handled gently using low-stress handling techniques, ensuring that no force or rough movements were applied. The environment was kept calm and quiet to create a stress-free atmosphere. Furthermore, cows were only inseminated when they showed clear signs of estrus, and the process was conducted swiftly to avoid prolonged handling, ensuring that the cows remained comfortable throughout the process. Artificial insemination was conducted in the 8th hour after the first appearance of estrus. The semen was deposited at the corpus uteri during AI. The frozen semen used in this study was supplied by the Singosari Artificial Insemination Center, and its quality has been previously documented by Yekti *et al.* (2023). Farmers reporting their cows exhibiting estrous behavior prompted the use of artificial insemination. Insemination was performed eight hours after the onset of estrus signals. Eight hours following the onset of estrus signals, the inseminator inseminated the cows. NRR-1 monitoring was carried out on days 19-22 following AI. Days 39-42 after AI, observation was followed by NRR-2 observation if the cow did not show estrus. Cows that did not show signs of estrus during NRR-1 and NRR-2 observations were confirmed pregnant using the rectal palpation method on day 60 after AI. Rectal palpation, an old long-used, and cost-effective method for diagnosis of pregnancy in cattle, involves manual examination of the uterus to detect the presence of a fetus. Although it provides rapid results, it requires a skilled practitioner and carries the risk of fetal damage (Jaśkowski *et al.*, 2019).

Non-return rate

Non-return rate 1 (NRR-1) was a metric used to evaluate the proportion of cows displaying signs of estrus during the first estrous cycle, specifically between days 19 and 22 post-AI. Non-return rate 2 (NRR-2) measured the percentage of cows that did not exhibit estrus during the second cycle, which occurred between days 39 and 42 after AI. According to Syah *et al.* (2024), NRR-1 and NRR-2 values were calculated using the following formulas.

$$\text{NRR1} = (\text{Total inseminated cows} - \text{total cows showing signs of estrus on days 19 to 22} / \text{Total inseminated cows}) \times 100$$

$$\text{NRR2} = (\text{Total inseminated cows} - \text{total cows showing signs of estrus on days 39 to 42} / \text{Total inseminated cows}) \times 100$$

Cows that did not show any signs of estrus during the NRR observations were assumed to be pregnant and were subsequently examined followed by a pregnancy examination using the rectal palpation method on day 60.

Conception rate

The conception rate (CR) represents the proportion of cows that became pregnant after the first insemination (Souames and Berrama, 2020). This parameter was used to assess the success of pregnancy after cows were considered pregnant based on NRR-1 and NRR-2 observations. Cows not showing signs of estrus at either observation time were subsequently checked for pregnancy using the rectal palpation method, performed on day 60 after AI. According to

Jainudeen and Hafez (2000), CR was calculated as follows: the number of pregnant cows from the first insemination is divided by the total number of cows used in the study multiplied by 100.

$$\text{CR (\%)} = (\text{Total cows pregnant at first insemination} / \text{Total inseminated cows}) \times 100$$

Statistical analysis

Data were tested statistically using R Studio version 4.3.3. This study used a chi-square test to compare the pregnancy success of the three treatments. Statistical significance was expressed at $p \leq 0.05$.

RESULTS AND DISCUSSION

The effect of different AI timings on NRR-1 and NRR-2 is shown in Table 1. No significant differences were found in NRR-1 and NRR-2 values between AI timings ($p > 0.05$). Although there was no significant difference, T2 showed the lowest NRR-1 and NRR-2 values, which were 80% and 66%, respectively. In the present study, a decrease in NRR-1 to NRR-2 values was observed across all treatments, with the largest drop in T2 (14%), indicating that the cows in T2 showed the most estrus signs over the two estrus cycles. In contrast, decreases in NRR-1 to NRR-2 in T1 and T3 were 11% and 10%, respectively.

The NRR-1 values for T1, T2, and T3 were 82%, 80%, and 89%, respectively. An imperfect NRR-1 value means that some cows exhibited signs of estrus in the first estrus cycle after AI. Furthermore, this is explained by the failure of fertilization between spermatozoa and ovum. Moreover, the failure of fertilization might arise from poor semen quality so spermatozoa penetration is not optimal. Alternatively, cows might experience stress, which might lead to reduced quality of the ovum (Walsh et al., 2011). For NRR-2, values were 71%, 66%, and 79%, respectively. There was a decrease in all treatments in the NRR-2 value, attributable to early embryonic death or silent heat (Syah et al., 2024). Early embryonic death can occur if the cow experiences a deficiency of progesterone hormone (Smith et al., 2022), as this hormone functions to maintain pregnancy, and insufficient levels can increase early embryonic mortality (Baruselli et al., 2022). Silent heat, where estrus signs are minimal or absent despite ovulation, can also affect NRR-2 (Sammad et al., 2020). The reduced NRR1 and NRR2 values observed in T2 could be attributed to elevated ambient temperatures. Temperatures in AI at midday (T2) can reach up to 29°C (Figure 1). High ambient temperatures increase the potential for cows to suffer from heat stress (Herbut et al., 2021). Heat stress is a condition in which certain mechanisms are triggered to maintain the body temperature of cattle when exposed to uncomfortably high temperatures (Dash et al., 2016). Heat stress conditions cause a decrease in the quality of spermatozoa in the female reproductive organs during penetration into the fallopian tubes. Spermatozoa exposed to high temperatures in the female reproductive tract can reduce spermatozoa viability, therefore disrupting fertilization and embryo development (Hansen et al., 2001). Furthermore, while high ambient temperatures may induce stress in cattle, studies have shown that their direct impact on ovulation is less pronounced compared to other reproductive processes (Roth, 2020). Nonetheless, heat stress significantly affects fertility rates and increases early embryonic death risks (Wolfenson and Roth, 2019).

The effects of different AI timings on CR are presented in Table 1. In this study, AI timing did not show a statistically significant difference in pregnancy success ($p > 0.05$). Although the difference between treatments indicated no significant difference, T2 showed the lowest pregnancy success rate, while T3 showed the highest CR value. Furthermore, there was a decrease in the percentage of NRR-2 to CR in all treatment groups. While no significant differences were observed, T2 had the lowest NRR-1, NRR-2, and CR values, indicating that insemination from 11:00 am to 3:59 pm may be associated with higher ambient temperatures compared to the other timings. Research on the effects of AI timing on conception rates in lactating Holstein-Friesian cows remains limited. This lack of research is likely due to several factors, including the historical focus on other reproductive technologies and the assumption that optimal insemination timing has been adequately addressed in standardized protocols. However, the timing of AI can significantly affect conception rates due to physiological variations in the estrous cycles of cows, influenced by factors such as hormonal changes, ambient temperature, and stress levels at different times of the day. Further research into AI timing is essential as understanding the optimal timing of insemination can improve conception rates, especially in lactating cows where reproductive efficiency is critical to maintaining milk production and fertility. Addressing these shortcomings will contribute to refining AI practices and increasing reproductive success in dairy farming operations, especially in areas with variable environmental conditions.

In this study, AI was conducted at the 8th hour after the onset of estrus. Therefore, the time of insemination was matched with the onset of estrus. Cows inseminated at 11:00 am–3:59 pm had a lower conception rate, although there was no significant difference between T1 and T3 ($p > 0.05$). Artificial insemination conducted during the midday (T2) showed no significant difference with AI in the morning (T1) and in the afternoon (T3), so AI can be conducted at any

time as long as the cows are still in the estrus phase. Previous studies have shown that the optimal time is 4–16 hours after estrus (López-Gatius, 2022; Udin et al., 2022).

Artificial insemination conducted more than 16 hours after the onset of estrus tends to have lower conception rates. However, this could be due to the time of ovulation being too close to the time of insemination, as estrus length is generally 24 to 33 hours and spermatozoa need 6-8 hours to capacitate (López-Gatius, 2022; De Rensis et al., 2024). Capacitation, a complicated process occurring in the reproductive organs of females, allows spermatozoa to bind to and penetrate the zona pellucida, facilitating fertilization (Mostek et al., 2021).

These results support previous studies suggesting that the timing between AI and ovulation is crucial for pregnancy success (Lauber et al., 2020). This study also confirms that AI can be done with the "a.m.-p.m. insemination rule", where cows showing estrus symptoms in the morning can be inseminated in the afternoon of the same day (Foote et al., 1979).

Table 1. Non-return rate and conception rate values in Holstein-Friesian cows at Sinau Andandani Ekonomi Cooperative Pujon, Malang, East Java, Indonesia

Variable	T1 (n=38)		T2 (n=82)		T3 (n=71)		P-value
	Not estrus	Pregnant	Not estrus	Pregnant	Not estrus	Pregnant	
NRR1	31 (82%)	-	66 (80%)	-	63 (89%)	-	0.35
NRR2	27 (71%)	-	54 (66%)	-	56 (79%)	-	0.20
CR	-	19 (50%)	-	39 (48%)	-	38 (54%)	0.76

T1: Artificial insemination in the morning (06.00 am - 10.59 am), T2: Artificial insemination in the midday (11.00 am - 03.59 pm), T3: Artificial insemination in the afternoon (04.00 pm - 08.59 pm), NRR1: Non-return rate 1, NRR2: Non-return rate 2, CR: Conception rate

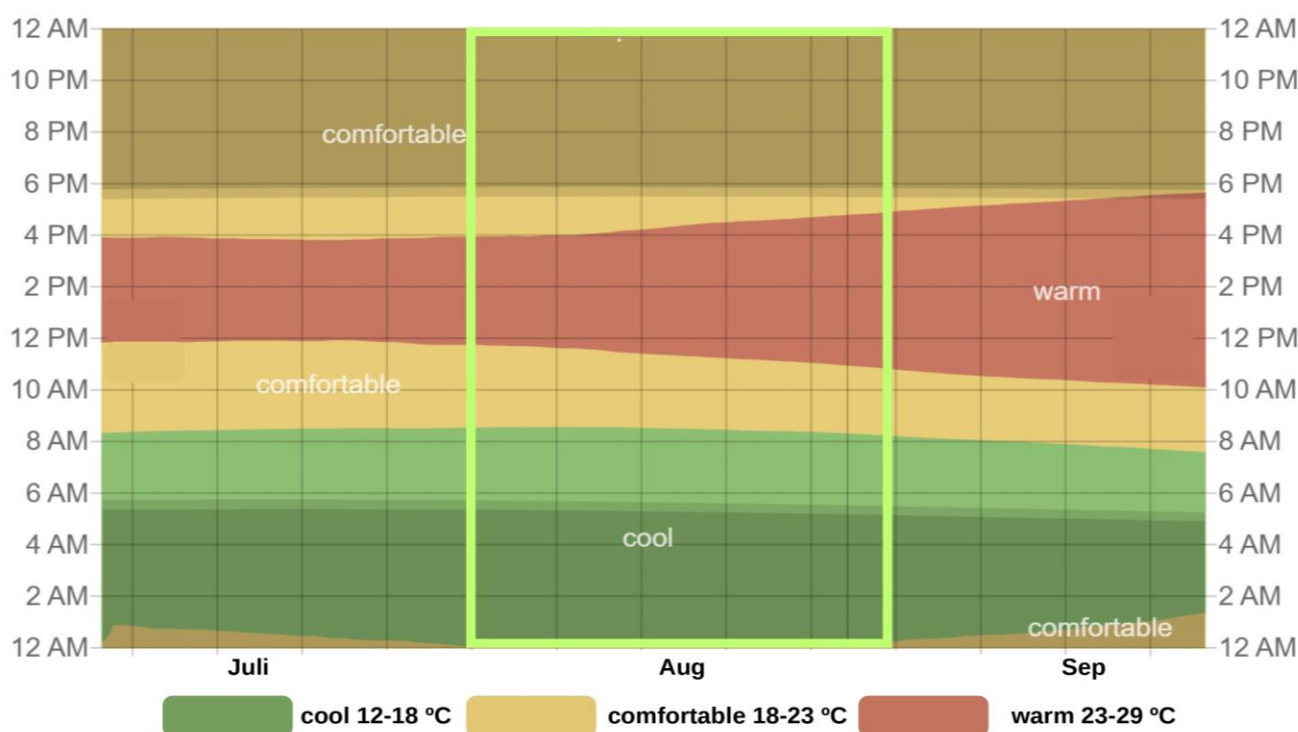


Figure 1. The hourly temperature at Sinau Andandani Ekonomi Cooperative Pujon, Malang, East Java, Indonesia, as recorded by weathers park

CONCLUSION

The timing of AI (morning, midday, and afternoon) in Holstein- Friesian cows did not affect pregnancy success. Although the difference in AI time did not show a significant effect, AI conducted in the midday had the lowest pregnancy success, while AI conducted in the afternoon showed the highest pregnancy success. While AI timing alone (morning, midday, or afternoon) did not significantly affect pregnancy success, further studies are recommended to incorporate additional environmental factors, such as temperature, humidity, and heat stress levels.

DECLARATIONS

Authors' contributions

Habib Asshidiq Syah wrote the manuscript, collected data, analyzed data, and reviewed the final version of the manuscript. Putri Utami reviewed the final version of the manuscript. Aulia Puspita Anugra Yekti, Nurul Isnaini, and Triril Susilawati designed the study, supervised the study, and reviewed the final version of the manuscript. All authors have approved the final edition of the manuscript manuscript.

Acknowledgments

The authors are grateful to the to the Direktorat Riset, Teknologi, dan Pengabdian kepada Masyarakat (DRTPM) for supporting this research under the Pendidikan Master Menuju Doktor untuk Sarjana Unggul (PMDSU) program.

Fundings

This research was funded by the Direktorat Riset, Teknologi, dan Pengabdian kepada Masyarakat (DRTPM) for supporting this research under the Pendidikan Master Menuju Doktor untuk Sarjana Unggul (PMDSU) program, contract number 006/E5/PG.02.00.PL.PMDSU/2024.

Ethical considerations

All authors have reviewed and confirmed the original content of the article before submission to this journal.

Availability of data and materials

The original contributions of this study are available within the article and its supplementary materials. For further information, please reach out to the corresponding authors.

Competing interests

The authors have not declared any conflict of interest.

REFERENCES

- Aitken RJ, Baker MA, and Nixon B (2015). Are sperm capacitation and apoptosis the opposite ends of a continuum driven by oxidative stress?. *Asian Journal of Andrology*, 17(4): 633-639. DOI: <https://www.doi.org/10.4103/1008-682X.153850>
- Am-in N, Tantasuparuk W, Manjarin R, and Kirkwood RN (2011). Effect of site of sperm deposition on fertility when sows are inseminated with aged semen. *Journal of Swine Health and Production*, 19(5): 295-297. DOI: <https://www.doi.org/10.54846/jshap/705>
- Baruselli PS, Catuzzi BLC, and de Abreu LÂ (2022). Use of reproductive biotechnologies to improve the fertility of repeat-breeder and heat-stressed dairy cows. *Spermova*, 12(1): 112-117. DOI: <https://www.doi.org/10.18548/aspe/0010.16>
- Dash S, Chakravarty AK, Singh A, Upadhyay A, Singh M, and Yousuf S (2016). Effect of heat stress on reproductive performances of dairy cattle and buffaloes: A review. *Veterinary World*, 9(3): 235-244. DOI: <https://www.doi.org/10.14202/vetworld.2016.235-244>
- De Rensis F, Dall'Olio E, Gnemmi GM, Tummaruk P, Andrani M, and Saleri R (2024). Interval from oestrus to ovulation in dairy cows-A key factor for insemination time: A review. *Veterinary Sciences*, 11(4): 152. DOI: <https://www.doi.org/10.3390/vetsci11040152>
- Diskin MG (2014). Achieving high reproductive performance in beef herds. Agriculture and food development authority. Grange, pp. 119-124. Available at: <https://www.teagasc.ie/media/website/animals/beef/High-reproductive-performance-in-beef-herd.pdf>
- Donnellan EM, Lonergan P, Meade KG, and Fair S (2022). An *ex-vivo* assessment of differential sperm transport in the female reproductive tract between high and low fertility bulls. *Theriogenology*, 181: 42-49. DOI: <https://www.doi.org/10.1016/j.theriogenology.2022.01.011>
- Fernandez-Novo A, Fargas O, Loste JM, Sebastian F, Perez-Villalobos N, Pesantez-Pacheco JL, Patron-Collantes R, and Astiz S (2020). Pregnancy loss (28-110 Days of Pregnancy) in Holstein cows: A retrospective study. *Animals*, 10(6): 925. DOI: <https://www.doi.org/10.3390/ani10060925>
- Foote RH, Oltenacu EAB, Mellinger J, Scott NR, and Marshall RA (1979). Pregnancy rate in dairy cows inseminated on the basis of electronic probe measurements. *Journal of Dairy Science*, 62(1): 69-73. DOI: [https://www.doi.org/10.3168/jds.S0022-0302\(79\)83204-X](https://www.doi.org/10.3168/jds.S0022-0302(79)83204-X)
- Hamid MA (2018). Study on the effect of insemination time on pregnancy rate of Bangladeshi buffalo in intensive farming. *Saarc Journal of Agriculture*, 16(2): 143-152. DOI: <https://www.doi.org/10.3329/sja.v16i2.40266>
- Hansen PJ, Drost M, Rivera RM, Paula-Lopes FF, Al-Katanani YM, Krininger CE, and Chase CC (2001). Adverse impact of heat stress on embryo production: Causes and strategies for mitigation. *Theriogenology*, 55(1): 91-103. DOI: [https://www.doi.org/10.1016/S0093-691X\(00\)00448-9](https://www.doi.org/10.1016/S0093-691X(00)00448-9)

- Hawk HW (1987). Transport and fate of spermatozoa after insemination of cattle. *Journal of Dairy Science*, 70(7): 1487-1503. DOI: [https://www.doi.org/10.3168/jds.S0022-0302\(87\)80173-X](https://www.doi.org/10.3168/jds.S0022-0302(87)80173-X)
- Herbut P, Hoffmann G, Angrecka S, Godyń D, Vieira FMC, Adamczyk K, and Kupczyński R (2021). The effects of heat stress on the behaviour of dairy cows—A review. *Annals of Animal Science*, 21(2): 385-402. DOI: <https://www.doi.org/10.2478/aoas-2020-0116>
- Hunter RHF and Greve T (1997). Could artificial insemination of cattle be more fruitful? Penalties associated with ageing eggs. *Reproduction in Domestic Animals*, 32(3): 137-141. DOI: <https://www.doi.org/10.1111/j.1439-0531.1997.tb01271.x>
- Jainudeen MR and Hafez ESE (2000). Cattle and buffalo in reproduction in farm animal. In: E. S. E. Hafez, and B. Hafez (Editors), *Reproduction in farm animals*. Lippincott Williams and Wilkins, pp. 157-171. DOI: <https://www.doi.org/10.1002/9781119265306.ch11>
- Jaśkowski JM, Kaczmarowski M, Kulus J, Jaśkowski BM, Herudzińska M, and Gehrke M (2019). Rectal palpation for pregnancy in cows: A relic or an alternative to modern diagnostic methods. *Medycyna Weterynaryjna*, 75(5): 259-264. DOI: <https://www.doi.org/10.21521/mw.6156>
- Lauber MR, McMullen B, Parrish JJ, and Fricke, PM (2020). Effect of timing of induction of ovulation relative to timed artificial insemination using sexed semen on pregnancy outcomes in primiparous Holstein cows. *Journal of Dairy Science*, 103(11): 10856-10861. DOI: <https://www.doi.org/10.3168/jds.2020-18836>
- López-Gatius F (2022). Revisiting the timing of insemination at spontaneous estrus in dairy cattle. *Animals*, 12(24): 3565. DOI: <https://www.doi.org/10.3390/ani12243565>
- Mahdavinezhad F, Gharaei R, Farmani AR, Hashemi F, Kouhestani M, and Amidi F (2021). The potential relationship between different human female reproductive disorders and sperm quality in female genital tract. *Reproductive Sciences*, 29: 695-710. DOI: <https://www.doi.org/10.1007/s43032-021-00520-7>
- Marques LR, de Almeida JVN, Oliveira AC, Paim TDP, Marques TC, and Leão KM (2024). Artificial insemination timing on pregnancy rate of Holstein cows using an automated activity monitoring. *Ciencia Rural*, 54(3): 1-5. <https://www.doi.org/10.1590/0103-8478cr20220557>
- Mostek A, Janta A, Majewska A, and Cierieszko A (2021). Bull sperm capacitation is accompanied by redox modifications of proteins. *International Journal of Molecular Sciences*, 22(15): 7903. DOI: <https://www.doi.org/10.3390/ijms22157903>
- Roelofs JB, Graat EAM, Mullaart E, Soede NM, Voskamp-Harkema W, and Kemp B (2006). Effects of insemination-ovulation interval on fertilization rates and embryo characteristics in dairy cattle. *Theriogenology*, 66(9): 2173-2181. DOI: <https://www.doi.org/10.1016/j.theriogenology.2006.07.005>
- Roth Z (2020). Influence of heat stress on reproduction in dairy cows—Physiological and practical aspects. *Journal of Animal Science*, 98(1): 80-87. DOI: <https://www.doi.org/10.1093/jas/skaa139>
- Sammad A, Umer S, Shi R, Zhu H, Zhao X, and Wang Y (2020). Dairy cow reproduction under the influence of heat stress. *Journal of Animal Physiology and Animal Nutrition*, 104(4): 978-986. DOI: <https://www.doi.org/10.1111/jpn.13257>
- Smith BD, Poliakiwski B, Polanco O, Singleton S, de Melo GD, Muntari M, Oliveira Filho RV, and Pohler KG (2022). Decisive points for pregnancy losses in beef cattle. *Reproduction, Fertility and Development*, 35(2): 70-83. DOI: <https://www.doi.org/10.1071/RD22206>
- Souames S and Berrama Z (2020). Factors affecting conception rate after the first artificial insemination in a private dairy cattle farm in North Algeria. *Veterinary World*, 13(12): 2608-2611. DOI: <https://www.doi.org/10.14202/vetworld.2020.2608-2611>
- Susilawati T (2014). Sexing Spermatozoa. UB Press, Malang, pp. 47-55. Available at: <https://fapet.ub.ac.id/wp-content/uploads/2017/10/Sexing-spermatozoa-bu-trinil.pdf>
- Syah HA, Yekti APA, Girinata IPA, Husen AF, Prafitri R, Isnaini N, Febrianto N, Utami P, Rifa'i M, and Susilawati T (2024). Evaluation of artificial insemination success of crossbred Friesian Holstein cow after foot and mouth disease outbreak. *Advances in Animal and Veterinary Sciences*, 12(7): 1249-1255. DOI: <https://www.doi.org/10.17582/journal.aavs/2024/12.7.1249.1255>
- Szenci O, Szelényi Z, Lénárt L, Buják D, Kovács L, Fruzsina Kézér L, Han B, and Horváth A (2018). Importance of monitoring the periparturient period to increase reproductive performance in dairy cattle. *Veterinárska Stanica*, 49(4): 297-307. Available at: <https://hrcak.srce.hr/file/325633>
- Tadesse B, Reda AA, Kassaw NT, and Tadeg W (2022). Success rate of artificial insemination, reproductive performance and economic impact of failure of first service insemination: A retrospective study. *BMC Veterinary Research*, 18(1): 226. <https://www.doi.org/10.1186/s12917-022-03325-1>
- Udin Z, Hendri H, and Masrizal M (2022). Increasing the success of artificial insemination through control of local cattle estrus as a genetic resource. *International Journal of Health Sciences*, 6(4): 2117-2132. <https://www.doi.org/10.53730/ijhs.v6nS4.6713>
- Walsh SW, Williams EJ, and Evans ACO (2011). A review of the causes of poor fertility in high milk producing dairy cows. *Animal Reproduction Science*, 123(4): 127-138. DOI: <https://www.doi.org/10.1016/j.anireprosci.2010.12.001>
- Wolfenson D and Roth Z (2019). Impact of heat stress on cow reproduction and fertility. *Animal Frontiers*, 9(1): 32-38. <https://www.doi.org/10.1093/af/vfy027>
- Yamada K, Nakao T, and Isobe N (2003). Effects of body condition score in cows periparturient on the onset of postpartum ovarian cyclicity and conception rates after ovulation synchronization/fixed-time artificial insemination. *Journal of Reproduction and Development*, 49(5): 381-388. Available at: <https://pdfs.semanticscholar.org/f220/6253dfae6044455d90146af6d1604eb7dd1a.pdf>
- Yekti APA, Rahayu S, Ciptadi G, and Susilawati T (2023). The quality and proportion of spermatozoa X and Y in sexed frozen semen separated with Percoll density gradient centrifugation method on Friesian Holstein bull. *Advances in Animal and*

Publisher's note: Scienceline Publication Ltd. remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access: This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <https://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2024