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# Conception Rate of Filial Friesian Holstein Cows After Being Inseminated Using Unsexed and Sexed Semen

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**ABSTRACT:** This research aimed to increase the reproduction of dairy cows with artificial insemination (AI) in Filial Friesian Holstein cows using sexed semen. This research was conducted in Pandesari Village, Pujon District, Malang Regency, East Java. A total 114 Filial Friesian Holstein cows were used in this research and divided into three Treatments: T1: 38 cows were inseminated using unsexed semen, T2: 38 cows were inseminated using albumin sedimentation sexed semen, and T3: 38 cows were inseminated using Percoll density gradient centrifugation (PDGC) sexed semen. The material was selected by purposive sampling with a minimum body condition score (BCS) specification of 2.5 (scale 1-5); the material had normal reproductive organs and showed signs of heat/estrus. The parameters of this study are the percentage of non-return rate (NRR) 1, NRR 2, and conception rate (CR). The data obtained were analyzed using descriptive analysis. The differences in NRR and CR between the unsexed sperm, sexed sperm with albumin sedimentation, and sexed sperm with PDGC were analyzed with the chi-square test and were considered significant at $p < 0.05$. The chi-square test was carried out to compare the observed values with the expected values. The results showed that the success of artificial insemination was greater by using albumin-sedimented sexed semen compared to unsexed semen or PDGC-sexed semen, with NRR values of 1 (95%), NRR values of 2 (87%) and CR values of 63%. The conception rate of artificial insemination using albumin-sedimented sexed semen was 63% greater than that of artificial insemination using unsexed semen and PDGC-sexed semen, which obtained the same value of 47%.

**Keywords:** Albumin sedimentation; Artificial insemination; Conception rate; Friesian Holstein; Sexed semen

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## INTRODUCTION

Filial Friesian Holstein cows are dairy cows resulting from the crossbreeding of Holstein Friesian (FH) cows with local cows (Siska et al., 2021). One way to support increasing the population and production of dairy cows is by applying reproductive biotechnology, such as artificial insemination (Utami et al., 2022). Artificial insemination is a reproductive technology that can be applied on smallholder farms to increase livestock productivity by utilizing superior male spermatozoa in the form of frozen semen, increasing the number of offspring more quickly and passing on good genetic traits. The success of AI involves many factors, including breed, semen quality, prospective breed stock, inseminator skills, time of AI, and detection of estrus from breeders (Susilawati et al., 2016).

Farmers in Pandesari Village, Pujon District, still apply AI to dairy cows using unsexed semen. The implementation of AI with the hope that the application of spermatozoa sexing technology can accompany the birth of female livestock in dairy cows. Using sexed semen for artificial insemination is one strategy to obtain replacement females for dairy cows (Lauber et al., 2020). The use of sexing spermatozoa is needed to identify and regulate the sex of calves. It is determined by the X and Y chromosomes (Bhalakiya et al., 2018). The sexing technologies widely used in Indonesia are albumin sedimentation sexing and PDGC sexing. Sexing using the albumin sedimentation method involves the separation of spermatozoa based on differences in motility or ability to move X and Y spermatozoa and differences in motility between X and Y spermatozoa due to differences in size and weight or mass. The motility of Y spermatozoa is faster than that of X spermatozoa due to the smaller mass and size of Y sperm than X sperm, which increases the motility of Y spermatozoa (Wahjuningsih et al., 2019).
Sexing spermatozoa using albumin is an easy method of separating spermatozoa that can produce between 71% and 76% of X and Y spermatozoa (Gunawan et al., 2015).

Sexing uses the PDGC method based on the difference in weight and head size of X and Y spermatozoa so that at the appropriate speed and time, X and Y spermatozoa can be separated. A cell separator material that is nontoxic to spermatozoa and can be used as a gradient (Promthep et al., 2016). The duration of the centrifugation process affects the quality of spermatozoa. A centrifugation time of 5 minutes resulted in a more significant proportion of X spermatozoa, namely, 78.6%, and spermatozoa motility, 57%, compared to 7 minutes, with a proportion of X spermatozoa, namely, 76.5%, and spermatozoa motility, 52.50% (Fatahillah et al., 2016).

The reproductive success of AI-produced cattle can be assessed by observing the NRR and CR parameters (Rosita et al., 2014). The success of AI using unsexed semen and sexed semen using the PDGC method had CR values of 44.44% and 25.92%, respectively (Wahyudi et al., 2014). The conception rates for single-dose AI results using unsexed semen and sexed PDGC semen were 35.48% and 43.47%, respectively (Setiyani et al., 2018). Knowing the NRR and CR values can determine the proper sexing method so that pregnancy success can increase.

## MATERIALS AND METHODS

This research was conducted in Pandesari Village, Pujon District, Malang, East Java. A total of 114 Filial Friesian Holstein cows were used in this research. Samples were selected by purposive sampling with a minimum BCS of 2.5-3.5 (scale 1-5) and were identified as having normal organs through rectal palpation and showing signs of heat/estrus. The PDGC sexed semen used was produced by the Singosari National Artificial Insemination Center (SNAIC), and the Lembang Artificial Insemination Center produced the albumin sedimentation sexed semen.

This study had three treatments: T1, 38 cows inseminated with unsexed semen; T2, 38 cows inseminated with albumin sedimentation sexed semen; and T3, 38 cows inseminated with PDGC sexed semen. The implementation of artificial insemination is carried out by inseminators when there is a report from the farmer that the livestock shows signs of heat. The inseminator inseminates with a single dose method at 8 hours after showing signs of heat/estrus (Kusuma et al., 2021). The thawing technique uses a temperature of 37°C for 30 seconds, and according to the Indonesian National Standard and Yekti et al. (2023), semen deposition is carried out at position four or in the corpus uteri. To increase pregnancy success, after AI is carried out, the acceptor is injected with 20 ml of vitamin ADE to increase immunity and minimize reproductive disorders because there is previous information that cows experience reproductive disorders (Agustina et al., 2020). The observed variables are NRR 1, NRR 2, and CR. The NRR is the percentage of cows that do not return to heat after insemination. This observation was carried out on days 19-22 (NRR 1) and 39-43 (NRR 2) after insemination. The conception rate is the percentage of pregnant cows resulting from the first insemination (Susilawati et al., 2022). These variables can be calculated using the following formula:

a. Non-Return Rate (NRR) $= \frac{Total\ inseminated\ cows - Total\ cows\ in\ heat/estrus}{Total\ inseminated\ cows}\ x\ 100\%$

b. Conception rate (CR) $= \frac{Total\ pregnant\ cows\ from\ first\ insemination}{Total\ inseminated\ cows}\ x\ 100\ \%$

The data obtained were analyzed using descriptive analysis. The differences in non-return rates and conception rates between the unsexed sperm, sexed sperm with albumin sedimentation, and sexed sperm with PDGC groups were analyzed with the chi-square test. It was considered significant at $p<0.05$. The chi-square test using the Statistical Package for the Social Sciences (SPSS) was carried out to compare the observed and expected values.

## RESULTS AND DISCUSSION

### Percentage of Non-Return Rate 1

The non-return rate (NRR) is one of the methods used for estimating pregnancy success after insemination (Malda et al., 2022).

**Table 1.** Non-Return Rate 1 of Filial Friesian Holstein Cows

| Treatments | Total AI | Total cows did not show heat | % Cows did not show heat | p-value |
|---|---|---|---|---|
| T1 | 38 | 29 | 76,00 | p>0.05 |
| T2 | 38 | 36 | 95,00 | p>0.05 |
| T3 | 38 | 31 | 82,00 | p>0.05 |
| Total | 114 | 96 | | |

Based on the research results shown in Table 1, it is known that the highest percentage of non-return rate 1 is T2, with a value of 95%, followed by T3, which is 82%, and T1, which is 76%. According to Setiyani et al. (2018), the NRR is considered good if it is between 70% and 90%. This means that the percentage of NRR in this study is optimal or good. The evaluation of the success of AI against NRR observations was guided by the fact that acceptors who do not show signs of heat within a certain period are assumed to be pregnant. NRR observations in this study were carried out on days 19–22 and 39–43. Acceptors that showed signs of heat during the NRR observation period after insemination were considered to have failed. Pearson chi-square analysis of the percentage of non-return rate one relative to the expected value

revealed no significant difference among the three treatments (P>0.05). The return of cows to heat can be caused by fertilization failure.

The factors that cause fertilization failure are very complex and include genetics, age, uterine infection, return of the estrous cycle, abnormal anatomy of the female reproductive tract, hormonal dysfunction, poor follicular development, and nutritional influences. Factors affecting fertilization failure include male fertility, semen quality, semen deposition during AI, and AI timing. One factor that affects the NRR is the maintenance management of cows, including feed. Cows that were given feed with additional concentrates showed an NRR value of 95%, while cattle that were reared without other feed additives had an NRR value of 80% (Saili et al., 2016).

**Percentage of Non-Return Rate 2**

The non-return rate (NRR) is the percentage of cattle that do not return to heat after being inseminated (Kusuma et al., 2021).

**Table 2.** Non-Return Rate 2 of Filial Friesian Holstein Cows

| Treatments | No. of AI | No. cows did not show heat | % Cows did not show heat | p-value |
|---|---|---|---|---|
| T1 | 38 | 24 | 63 | p<0.01 |
| T2 | 38 | 33 | 87 | p>0.05 |
| T3 | 38 | 28 | 74 | p<0.05 |
| Total | 114 | 85 | | |

Based on the research results shown in Table 2, it is known that the highest percentage of non-return rate 2 is T2, with a value of 87%, followed by T3, which is 74%, and T1, which is 63%. The results of the observations of NRR 2 showed a decrease in the percentage of NRR 1. The reduction in T1 was 13%, that in T2 was 8%, and that in T3 was 8%. The results of this study indicated that the NRR percentage of frozen semen sexing was greater than that of nonsexing frozen semen. The NRR resulting from albumin sedimentation is higher because the sexing process can separate live and dead spermatozoa, so sexing by albumin sedimentation can increase pregnancy.

Insemination using sexed semen in cows is recommended to maximize the pregnancy rate in cows, especially in dairy cows. Insemination during oestrus aims to increase the pregnancy rate in cows (Guner et al., 2022). During oestrus, cows show signs such as a red vulva, swollen vulva, and mucus discharge from the cervix. It occurs due to the hormone estrogen in the peak phase (Utami et al., 2022). Spermatozoa that have high fertility then have high commercial value. The fertility rate determines the service per conception to determine pregnancy (Wahjuningsih et al., 2019). The use of sexed semen by Susilawati (2019) does not always result in a pregnancy, as expected. Artificial insemination using double-dose sexed semen had a high success rate.

Pearson chi-square analysis of the percentage of non-return rate two plants with an expected value revealed a very significant difference at T1 (P<0.01), no significant difference at T2 (P>0.05), and a significant difference at T3 (P<0.05). The decrease in the percentage of NRR 1 to NRR 2 occurred due to the increase of female cows that experience estrous. Cows subjected to heat are characterized by swollen and reddened vulvas, decreased milk production, and clear mucus discharge from the vulva (Pemayun et al., 2014). However, it differs from a cow experiencing silent heat.

Cows in mild heat do not show signs of heat but have normal reproductive cycles; if signs of heat are lacking or not visible, the oestrus cycle will be missed from observation, and this condition is called silent heat. During the study, it was found

that several acceptors experienced silent heat, so farmers could not detect cows in heat.

The ovulation process in cases of silent heat commonly occurred, and no signs of heat appeared. Due to the low estrogen level, the requirements for the appearance of signs of heat are not fulfilled (Yekti et al., 2017). Silent heat can also be caused by ectoparasitic disorders, such as fleas found on the vulva, which can cause stress (Utami et al., 2022). In addition to silent heat, early embryonic death can cause a decrease in the NRR because embryos that fail to attach to the uterine wall will experience pregnancy failure (Yekti et al., 2017).

**Percentage of Conception Rate**

The CR is the percentage of acceptors who successfully conceived at the first known IB after carrying out a pregnancy examination. Costa et al. (2016) reported that CR can be used as an indicator of the fertility of female cows. The calculation of the CR in this study was based on a pregnancy examination using the rectal palpation method on the 60 to 90 days afterinsemination.

**Table 3.** The Conception Rate of Filial Friesian Holstein Cows

| Treatments | No. of AI | No. of Pregnant Cows | % of Pregnant Cows | p-value |
|---|---|---|---|---|
| T1 | 38 | 18 | 47 | p<0.01 |
| T2 | 38 | 24 | 63 | p<0.01 |
| T3 | 38 | 18 | 47 | p<0.01 |
| Total | 114 | 60 | | |

Based on the research results shown in Table 3, T2 had the highest percentage of conception rate, at 63%, followed by T1 and T3, who achieved the same percentage of success, 47%. Compared with that of unsexed semen, artificial insemination using albumin sedimentation sexed semen was more successful. The sexed semen used had a higher proportion of X spermatozoa than Y. Susilawati et al. (2019) stated that the duration of the encounter between sperm and egg was lengthy. Hence, those that could survive and be motile were X spermatozoa.

Pearson chi-square analysis of the percentage of conception rate relative to the expected value revealed a highly significant difference among the three treatments (P<0.01). The success percentage of T2 from this study can still be considered suitable for dairy cattle in Indonesia because, taking into account management, natural conditions, and distribution of livestock that are spread out, a CR value that reaches 45-50% can be said to be good. The success rate of pregnancy in this study was greater for cattle inseminated using sexing semen than for those inseminated using unsexed semen. Descriptively, the percentage of CR in this study is much different from that in the NRR estimation. One of the influencing factors is a decrease in the quality of the semen used for insemination. Decreasing the quality of spermatozoa after thawing can reduce the ability to fertilize and affect embryo development. In addition, an increase in the percentage of abnormal spermatozoa during the cooling and freezing process was caused by cold stress, an imbalance in osmotic pressure due to metabolic processes that continued during storage (Khalil et al., 2018).

Physiological conditions in livestock determine the mechanism of reproduction, production, growth, health, and quality of milk produced. The stress experienced by cows is due to the conditions and structure of the enclosure, which includes the availability of ventilation, floors, grouping of livestock, and the density of livestock in a cage, as well as weather that is too hot and too cold (Utami et al., 2022). All of these components can affect the performance of

the endocrine system, thereby affecting the reproductive system and the oestrus cycle in cows.

Heat stress can impair reproductive performance by reducing the duration and intensity of oestrus. In addition, a hot environment interferes with embryo development (Oikawa et al., 2019). Cows that experience stress can reduce their appetite, impact reproductive hormones, and decrease production (Setiawan et al., 2014). The success rate of artificial insemination in this study can be influenced by the quality of the semen used. The use of sexing semen in this study (T2) had the highest success rate (Table 3).

The quality of spermatozoa can be affected by several treatments in the centrifugation process of sexing semen, which can separate normal and dead spermatozoa so that only normal spermatozoa are left behind, and the NRR and CR values can increase. The centrifugation treatment during sexing can also damage the spermatozoa membrane, so cell organelles such as mitochondria and lysosomes will also be disrupted (Kusumawati et al., 2017).

Handling of sexed semen during processing must be done correctly because spermatozoa are very susceptible to changes in temperature, which will change their motility. An appropriate diluent is needed to help protect spermatozoa membranes from damage during the cooling and freezing processes. The diluent must contain nutrients, buffers, and cold shock (Wiratri et al., 2014). In addition, liquid nitrogen is also needed in frozen semen to maintain the quality of spermatozoa (Wahyudi et al., 2014).

## CONCLUSION

According to the study results, it can be inferred that AI success rates using sexed semen subjected to albumin sedimentation was greater than that of AI using unsexed semen and Percoll density gradient centrifugation of sexed semen according to the NRR 1, NRR 2 and CR values. The conception rate of artificial insemination using albumin-sedimented sexed semen was 63% greater than that of artificial insemination using unsexed semen and Percoll density gradient centrifugation sexed semen, which obtained the same value of 47%.

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## REFERENCES

Agustina, G. C., Hendrawan, V. F., Wulansari, D., & Oktanella, Y. (2020). Upaya Peningkatan Produksi Susu Sapi Perah Dengan Pemberian Vitamin Ade dan Obat Cacing. *Jurnal Nutrisi Ternak Tropis*, 3(1), 1-6. https://doi.org/10.21776/ub.jnt.2020.003.01.1

Bhalakiya, N., Haque, N., Patel, D., Chaudhari, A., Patel, G., Madhavatar, M., & Kumar, R. (2018). Sperm sexing and its application in livestock sector. *International Journal of Current Microbiology and Applied Sciences*, 2018(Special 7), 259-272.

Costa, N. D., Susilawati, T., Isnaini, N., & Ihsan, M. N. (2016). The difference of artificial insemination successful rate of onggole filial cattle using cold semen with different storage time with tris aminomethane egg yolk dilution agent. IOSR *Journal of Pharmacy*, 6(6), 13-19.

Fatahilah, F., Susilawati, T., & Isnanini, N. (2017). Pengaruh lama sentrifugasi terhadap kualitas dan proporsi spermatozoa xy sapi limousin hasil sexing dengan gradien densitas percoll menggunakan pengencer cep-2+ 10% kt. *TERNAK TROPIKA Journal of Tropical Animal Production*, 17(1), 86-97.

Gunawan, M.., Kaiin, E. M., & Said, S. (2015). Aplikasi inseminasi buatan

dengan sperma sexing dalam meningkatkan produktivitas sapi di peternakan rakyat. *Pros Sem Nas Masy Biodiv Indon*, 1(1), 93-96.. https://doi.org/10.13057/psnmbi/m010115

Guner, B., Erturk, M., Dursun, M., Ozturk, B., Yilmazbas-Mecitoglu, G., Keskin, A., & Gumen, A. (2023). Effect of oestrous expression prior to timed artificial insemination with sexed semen on pregnancy rate in dairy cows. *Reproduction in Domestic Animals,* 58(2), 342-348. https://doi.org/10.1111/rda.14293

Khalil, W. A., El-Harairy, M. A., Zeidan, A. E., Hassan, M. A., & Mohey-Elsaeed, O. (2018). Evaluation of bull spermatozoa during and after cryopreservation: Structural and ultrastructural insights. International Journal of Veterinary Science and Medicine, 6, S49-S56. https://doi.org/10.1016/j.ijvsm.2017.11.001

Kusuma, H. R., Huda, A. N., Prafitri, R., Yekti, A. P. A., & Susilawati, T. (2021). Evaluasi tingkat keberhasilan Inseminasi Buatan double dosis (jam ke 2 dan ke 8) terhadap kualitas berahi pada sapi persilangan Ongole. Jurnal Ilmu Ternak Universitas Padjadjaran, 21(2), 94-101. https://doi.org/10.24198/jit.v21i2.33594

Kusumawati, E. D., Isnaini, N., Yekti, A. P. A., Luthfi, M., Affandhy, L., Pamungkas, D., & Rahayu, S. (2017). The quality of sexed semen on filial ongole bull using percoll density gradient centrifugation method. Asian J. Microbiol. Biotechol Environ. Sci, 19, 189-199.

Lauber, M. R., McMullen, B., Parrish, J., & Fricke, P. (2020). Short communication: Effect of timing of induction of ovulation relative to timed artificial insemination using sexed semen on pregnancy outcomes in primiparous Holstein cows. J. Dairy Sci, 103(11), 10856-10861. https://doi.org/10.3168/jds.2020-18836

Malda, Y., Layla, N., Yekti, A. P. A., Huda, A. N., Kusmartono, K., & Susilawati, T. (2022). Pengaruh pemberian konsentrat pada waktu yang berbeda terhadap keberhasilan inseminasi buatan pada sapi Persilangan Limousin. Livestock and animal research, 20(3), 243-250. https://doi.org/10.20961/lar.v20i3.58662

Oikawa, K., Yamazaki, T., Yamaguchi, S., Abe, H., Bai, H., Takahashi, M., & Kawahara, M. (2019). Effects of use of conventional and sexed semen on the conception rate in heifers: A comparison study. Theriogenology, 135, 33-37. https://doi.org/10.1016/j.theriogenology. 2019. 06. 012

Pemayun, T. G. O., Trilaksana, I. G. N. B., & Budiasa, M. K. (2014). Waktu inseminasi buatan yang tepat pada sapi Bali dan kadar progesteron pada sapi bunting. Jurnal Veteriner, 15(3), 425-430.

Promthep, K., Satitmanwiwat, S., Kitiyanant, N., Tantiwattanakul, P., Jirajaroenrat, K., Sitthigripong, R., & Singhapol, C. (2016). Practical use of percoll density gradient centrifugation on sperm sex determination in commercial dairy farm in Thailand. Indian Journal of Animal Research, 50(3), 310-313. https://doi.org/10.18805/ijar.8427

Rosita, E. A., Susilawati, T., & Wahyuningsih, S. (2014). Keberhasilan IB menggunakan semen beku hasil sexing dengan metode sedimentasi putih telur pada sapi PO cross. Jurnal Ilmu-Ilmu Peternakan, 24(1), 72-76.

Saili, T., Rahadi, S., Sura, I. W., & Lopulalan, F. (2016). Sinkronisasi Estrus dan Inseminasi Buatan Menggunakan Semen Cair Hasil Sexing pada Sapi Bali Induk Yang Dipelihara dengan Sistem yang

Berbeda (Oestrus Syncronization and Artificial Insemination using Sexing Semen from Bali's Cattle with Different Management System). Jurnal Ilmu Ternak Universitas Padjadjaran, 16(2).

Setiawan, R. (2014). Studi AsosiasiAntara Masa Kosong (Days Open) Terhadap Produksi Susu dan Kerugian Ekonomi Pada Peternakan Sapi Perah Di Kabupaten Garut (Association study of days open on milk production and its implication on economic losses of dairy cattle in Garut Regen. Jurnal Ilmu Ternak Universitas Padjadjaran, 14(1).

Setiyani, D. S., Yekti, A. P. A., Kuswati, K., & Susilawati, T. (2018). Keberhasilan inseminasi buatan menggunakan semen sexing beku pada Sapi Persilangan Ongole. Jurnal Ilmu-Ilmu Peternakan, 28(3), 259-264. https://doi.org/10.21776/ub.jiip.2018.028.03.09

Siska, I., & Anggrayni, Y. L. (2020). Body Condition Score (BCS), Tingkat Laktasi dan Hubungannya dengan Produksi Susu Sapi Perah Peranakan Friesian Holstein (PFH). Jurnal Ilmu Ternak Universitas Padjadjaran, 20(2), 115-125. https://doi.org/10.24198/jit.v20i2.30922

Susilawati, T., Isnaini, N., Yekti, A. P. A., Nurjannah, I., & Errico, E. (2016). Keberhasilan inseminasi buatan menggunakan semen beku dan semen cair pada sapi Peranakan Ongole. Jurnal Ilmu-Ilmu Peternakan (Indonesian Journal of Animal Science), 26(3), 14-19.

Susilawati, T. (2019). Klaster Sapi Potong. Universitas Brawijaya Press, Malang. ISBN: 978-602-432-739-2.

Susilawati, T., Ihsan, M. N., Wahjuningsih, S., Isnaini, N., Rachmawati, A., Yekti, A. P. A., & Utami, P. (2022). Manajemen Reproduksi dan Inseminasi Buatan. Universitas Brawijaya Press. ISBN: 978-623-296-624-6.

Utami, P., Hanif, M. Z., Yekti, A. P. A., Prafitri, R., Huda, A. N., Kuswati, K., & Susilawati, T. (2022). Evaluation the success of artificial insemination using frozen sexed semen based on different estrus characters. Jurnal agripet, 22(2), 190-196. https://doi.org/10.17969/agripet.v22i2.23423

Utami, P., Hanif, M. Z., Yekti, A. P. A., Riyanto, J., Hanim, C., & Susilawati, T. (2022). The relationship of estrus character before double dose artificial insemination in Limousin Crossbred cow. In E3S Web of Conferences (Vol. 335, p. 00031). EDP Sciences. https://doi.org/10.1051/e3sconf/202233500031

Wahjuningsih, S., Susilawati, T., Ihsan, M. N., Busono, W., Isnaini, N., & Yekti, A. P. A. (2019). Teknologi Reproduksi Ternak. Universitas Brawijaya Press. ISBN: 978-602-432-843-6.

Wahyudi, L., Susilawati, T., & Isnaini, N. (2014). Tampilan reproduksi hasil inseminasi buatan menggunakan semen beku hasil sexing pada sapi persilangan ongole di peternakan rakyat. *TERNAK TROPIKA Journal of Tropical Animal Production*, 15(1), 80-88..

Bunga, V. D., Susilawati, T., & Wahjuningsih, S. (2014). Kualitas semen sapi Limousin pada pengencer yang berbeda selama pendinginan. *TERNAK TROPIKA Journal of Tropical Animal Production*, 15(1), 13-20.

Yekti, A. P. A., Susilawati, T., Ihsan, M. N., & Wahyuningsih, S. (2017). Fisiologi Reproduksi Ternak: Dasar Manajemen Reproduksi. Universitas Brawijaya Press. ISBN: 978-602-432-245-8.

Yekti, A. P. A., Setiawan, R. E. R., Rachmawati, A., & Susilawati, T. (2023). Kualitas Semen Beku Sapi

Limousin setelah Thawing Menggunakan Air Dingin dengan Lama Waktu yang Berbeda. Jurnal Agripet, 23(1), 25-32.