

Evaluation of Superovulation Results for In Vivo Embryo Production in Limousin and Simmental Cattle

Solana Muhamad Soleh¹, Rismasari¹, Dwi Wijayanti^{1*}, Novia Rahayu¹, Fahrudin Darlian², Putri Indah Ningtias²

¹*Department of Animal Science, Perjuangan University of Tasikmalaya, Tasikmalaya, West Java, 46115, Indonesia*

²*Cipelang Livestock Embryo Center, Bogor, Indonesia.*

*Corresponding E-mail : wijayantidwil2@gmail.com

ABSTRACT

This study aims to superovulation response and embryo quality of two cattle groups studied, namely the Limousin and Simmental cattle groups so that it can be known from the two cattle groups that have the best response level and embryo quality. This study was conducted at the Cipelang Livestock Embryo Center (BET), using secondary data on the quality and number of embryos produced in Limousin and Simmental donor cattle. The parameters observed in this study were the number of corpus luteum (CL), response rate, recovery rate, number of embryos collected (1, 2, 3, 4 (DG / UF)), and the proportion of transferable embryos, the proportion of degenerative embryos, and the proportion of unfertilized. The data collected were tested using the Mann-Whitney test. The results of this study, the number of CL, response rate, recovery rate, total embryos collected, the proportion of transferable embryos, the proportion of DG embryos, and the proportion of UF Limousin and Simmental cattle showed different results but were not significantly different ($P > 0.05$). This study concludes that Limousin cattle produce better embryo quality than Simmental cattle because they produce a higher number of CL, response rate, recovery rate, total embryos collected, and a lower proportion of degenerative embryos.

Keywords: Embryo, Limousin, Simmental, Superovulation.

INTRODUCTION

The development of beef in the last five years has decreased in the Java region by around 0.35% each year, but outside Java, it has increased by 2.33% each year (Revi *et al.*, 2018). In 2022, meat production in Indonesia reached 524.76 thousand tons (BPS, 2023). However, this amount is not comparable to meat consumption which reached 816.79 thousand tons (BPS, 2023). So Indonesia still experiences a shortage of meat production of $\pm$ 242.03 thousand tons to meet the consumption needs of the Indonesian people. The lack of meat production that Indonesia still experiences

every year is due to the lack of beef cattle population, in 2022 beef cattle also decreased to 0.73 million (BPS, 2023). Limousin and Simmental cattle are types of *Bos taurus* beef cattle that are quite widely raised by Indonesian farmers because these cattle have many advantages as a type of beef cattle. Limousin cattle have a high fertility rate, give birth easily, can breastfeed, and take good care of their calves. Meanwhile, Simmental cattle choose a very fast growth performance, in one day Simmental cattle can gain weight of around 0.6 - 1.5 kg so that when they are adults their body weight can reach 1,400 kg (Hartati *et al.*, 2022). The advantages of Limousin and Simmental

cattle are one of the reasons for the types of beef cattle that need to be improved because these two groups of cattle if developed in a large population, can help overcome the lack of beef production, this improvement can be done by improving genetic quality first by implementing superovulation reproductive biotechnology.

Superovulation is a treatment given to donor cows to produce more eggs than their natural conditions by providing certain hormonal treatments (Setiawan *et al.*, 2017). Thus, through superovulation activities, seeds with superior genetic quality will be produced, and through these seeds, a greater population increase will be produced with superior genetic quality so that the fulfillment of national seed needs can be overcome. Simmental and Limousin cows in previous studies had the same superovulation response, but Simmental cows produced more CL and DG quality embryos than Limousin cows, besides that, for the number of LT and UFO quality embryos, the two types of cows showed very different results (Liamanu *et al.*, 2018).

This study was conducted to determine the superovulation response, the comparison of the number of CL and embryos produced, and the proportion of embryos produced based on the quality of the Limousin and Simmental cattle herds. Thus, an evaluation can be carried out to improve the superovulation response or the quality of embryos produced by the two herds of cattle.

MATERIALS AND METHODS

Research Location and Materials

This research was carried out at the Cipelang Livestock Embryo Center (CLEC) located in Cipelang Village, Cijeruk District, Bogor Regency, West Java, Indonesia. This study was conducted using data on the quality and number of Limousin and Simmental donor cow embryos obtained from the Cipelang Livestock Embryo Center (BET) during the implementation of the superovulation program from 2020 to October 2023.

The procedure for performing superovulation in CLEC from the initial selection of donor cows to the storage of embryos produced is as follows (Figure 1).

The data obtained were the number of Limousin donor cows as many as 71 and Simmental as many as 74 in the superovulation program and donor cows that responded to superovulation, the number of Corpus Luteum (CL), the number of embryos collected, embryo quality (1, 2, 3, 4 (DG/UF)), with an average age of 5 years.

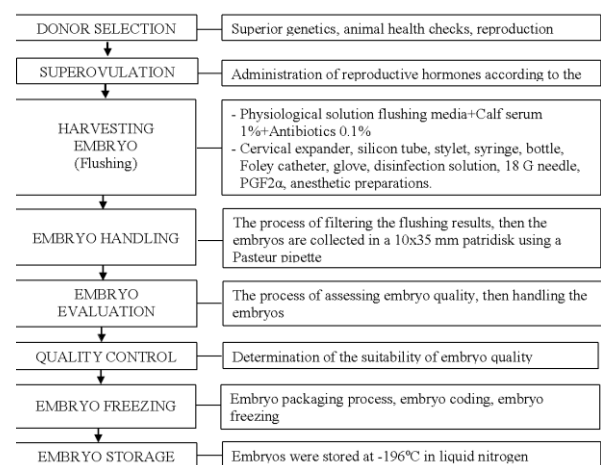


Figure 1. Steps in the superovulation program and in vivo embryo production, starting from donor selection to embryo storage.

Illustrates the steps involved in implementing the donor cows superovulation program and in vivo embryo production (Figure 2).

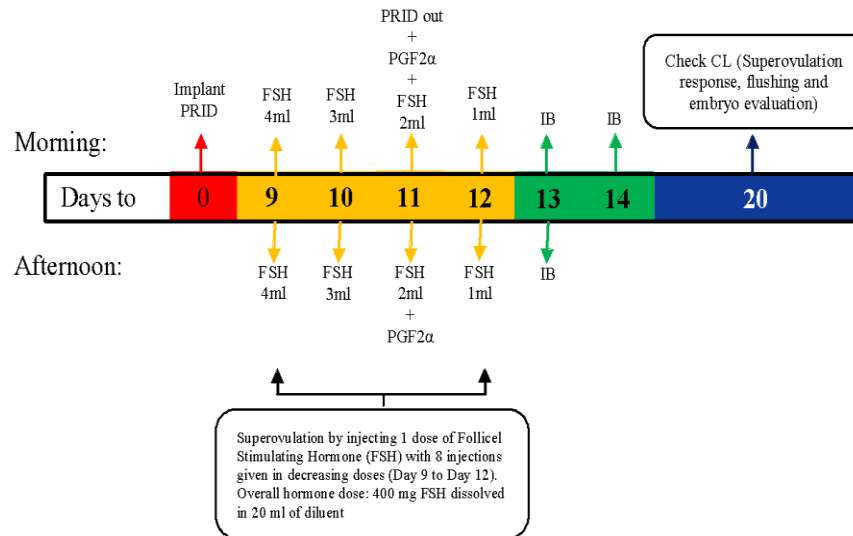


Figure 2. Superovulation Procedure. Day 0, installation of a progesterone device for follicular wave synchronization. Day 9 to day 12 of the superovulation program with FSH injection using a reduced dose. Day 11, synchronize lust by releasing the progesterone device and injecting the PGF2a hormone. Day 13 and 14 artificial insemination 3 times. Day 20 of rinsing.

Synchronization of Follicular Waves and Superovulation

Follicle wave synchronization is carried out by inserting a progesterone device (Cue-mate) intravaginally from day 0 to day 11 (Hardiyanto *et al.*, 2016). The superovulation program is carried out by injecting FSH on days 9 to 12 with decreasing dosage (4 ml, 3 ml, 2 ml, 1 ml in the morning and evening) intramuscularly (Table 1).

Table 1. Superovulation Treatment Schedule

Day to	Superovulation Treatment
0	Implant <i>Cue-mate</i> (Intra vagina)
9	Morning : FSH injection 4 ml (<i>intramuscular</i>) Afternoon: FSH injection 4 ml (<i>intramuscular</i>)

10	Morning: FSH injection 3 ml (<i>intramuscular</i>) Afternoon: FSH injection 3 ml (<i>intramuscular</i>)
11	Morning: Cue-mate removal + 2 ml FSH injection + PGF2a (<i>intramuscular</i>) Afternoon: 2 ml FSH + PGF2a injection (<i>intramuscular</i>)
12	Morning: 1 ml FSH injection (<i>intramuscular</i>) Afternoon: 1 ml FSH injection (<i>intramuscular</i>)
13	Morning: Artificial Insemination (IB) Afternoon: Artificial Insemination (IB)
14	Pagi : Inseminasi Buatan (IB)
20	Morning: CL check with rectal palpation (Superovulation response), flushing and embryo evaluation

Synchronization of Oestrus and Artificial Insemination (AI)

Lust synchronization is carried out by injecting the PGF2 α hormone exactly on the 11th day after the progesterone device implant (follicle wave synchronization). This PGF2 α hormone injection is carried out simultaneously with the removal of the progesterone device (cue-mate) and FSH injection on the 3rd day precisely when the FSH dose of 2 ml is given (Darlian *et al.*, 2021).

The implementation of artificial insemination (AI) for the superovulation program is carried out three times (morning, afternoon, morning) (Darlian *et al.*, 2021), but if the donor cow still shows signs of heat after 3 artificial inseminations (AI), then artificial insemination (AI) may be carried out again.

Embryo Flushing

At the Cipelang Livestock Embryo Center (BET), embryo harvesting is carried out on the 7th day after the implementation of AI, embryo harvesting is carried out by flushing the left, right, and body uterus 3 times alternately, using 500 ml of ringer lactate fluid each supplemented with 1% calf serum and antibiotics. So one cow will use up 1,500 ml of uterine flushing fluid which is collected in a flushing container bottle. Then the uterine flushing fluid that is already in the container bottle will be taken to the laboratory for embryo evaluation. After the uterine flushing process is complete, the next step is that the livestock will be given 50 ml of 2% iodine which is given

intravaginally which aims to minimize infection. After that, the livestock will be injected with the PGF2 α hormone to shed CL to avoid pregnancy and help the reproductive recovery process to be faster (Darlian *et al.*, 2021).

Embryo Evaluation

The embryo evaluation process can be started by filtering the flushing media using an embryo filter, embryo search is done on the embryo filtering media under a microscope with 40x magnification. Then the embryo will be evaluated based on its quality, the following are the criteria for determining embryos based on their quality (Table 2):

Table 2. Embryo Quality Assessment

Embryo Quality	Description
Quality 1 (A): Excellent or Good	1. The shape of the embryo is symmetrical and round (spherical) with blastomeres that are uniform in size, color, and density. 2. The embryo must have a shape consistent with the estimated developmental phase of the embryo itself. Relatively minor irregular shape. 3. Have a minimum of 85% intact cellular material and live embryo mass. 4. The zona pellucida should be round, smooth, not sticking to the petri dish or pipette.
Quality 2 (B): Fair	1. In general, it has an irregular shape in the medium category in terms of embryo mass, size, color and density of individual cells. 2. Have intact cells and live embryo mass of at least 50%

Quality 3 (C): Poor	1. Embryos are predominantly irregular in shape, size, color and density of individual cells. 2. Have intact cells and a live embryo mass of at least 25%.
Degenerative	Degenerative embryos are embryos that were once alive but died or did not develop according to their phase
Unfertilized	Unfertilized embryos are embryos that are not fertilized because when fertilization occurs the egg cells have not yet matured.

Research Parameters

1. Number of Corpus Luteum (CL)
2.
$$\text{Response Rate} = \frac{\text{Number of responding donor cows}}{\text{Number of donor cows in superovulation}} \times 100\%$$
3.
$$\text{Recovery Rate} = \frac{\text{Total number of embryos collected}}{\text{The number of CL formed in the ovaries}} \times 100\%$$
4. Embryo Collection
5.
$$\text{Transferable Embryos} = \frac{\text{Number of transferable embryos}}{\text{Number of embryos collected}} \times 100\%$$
6.
$$\%DG = \frac{\text{Number of DG embryos}}{\text{Number of embryos collected}} \times 100\%$$
7.
$$\%UF = \frac{\text{Number of UFO}}{\text{Number of embryos collected}} \times 100\%$$

Statistical Analyses

The number of corpus luteum (CL), response rate, recovery rate, total embryos collected, proportion of transferable embryos, proportion of degenerative embryos (DG), proportion of unfertilized ova (UFO), will be analyzed using the Mann-Whitney U Test with a significance level of ($P < 0.05$) using IBM SPSS 23 software.

RESULTS AND DISCUSSIONS

Number of Corpus Luteum (CL)

The corpus luteum (CL) is one of the benchmarks used to determine the response of donor cows to superovulation treatment after administration of the gonadotropin hormone, namely the follicle-stimulating hormone (FSH). The success of superovulation treatment can be seen from the ovarian response measured by the number of CLs formed due to the ovulation process from mature follicles (de Graaf). The more CL formed, the higher the response of the superovulation program (Subchan et al., 2016).

The number of CLs of Limousin and Simmental cattle in this study showed no significant difference ($P > 0.05$) (Table 3).

Table 3. Number of *Corpus Luteum* (CL)

Cattle Herd	Number CL	<i>p-value</i>
Limousin	9,48±7,15	0,20
Simmental	8,50±6,99	

This is because the concentration of FSH hormone given to Limousin and Simmental cattle in the superpopulation program was not different. The results of this study differ from the Liamanu *et al.* (2018) study which stated that, Simmental cattle produce a higher number of CLs than Limousin cattle, so the results of the study showed a very real difference between the number of CLs of Simmental and Limousin cattle which could be caused by differences in livestock groups.

Simmental cattle showed a lower average value of 8.50 while Limousin cattle produced a higher average CL of 9.48 (Table 3).

This is estimated because the number of CL levels from each cattle group is different. The average number of CL of Simmental cattle in this study was lower than in the Djunaedi *et al.* (2019) study, which was 11. The average number of CL of Limousin cattle in the Setiawan *et al.* (2017) study was also much larger, which was 13.4.

Response Rate

The response rate is calculated by comparing the number of livestock that responded to superovulation with the total number of livestock given superovulation treatment (Darlian *et al.*, 2021). In this study, cows were declared to have responded to the superovulation if they had a CL ≥ 3 .

The response rate of Limousin and Simmental cattle in this study showed results that were not significantly different ($P > 0.05$) (Table 4).

Table 4. Respon Rate

Cattle Herd	Respon Rate	p-value
Limousin	90,00 $\pm$ 30,21	0,12
Simmental	84,21 $\pm$ 36,70	

The absence of differences in response rates can be caused by several factors, one of which is the superovulation method used or the determination of the FSH injection time in the same superovulation program. The superovulation method used in this study was to inject FSH intramuscularly into the body of the livestock as much as 1 dose with 8 injections for four days (Days 9 to 12) in the morning and evening with decreasing doses. The injection

method is carried out because the half-life of the FSH hormone to survive in the body is only 2-5 hours, so the injection needs to be repeated for 4-5 days at intervals of 10-12 hours (Tjahjani *et al.*, 2021). Several factors influence the response rate or superovulation response in donor cows, including the donor cow seeds used, the age of the donor cows, the number of cattle used as donors, and the BCS of the donor cows (Liamanu *et al.*, 2018).

Recovery Rate

The calculation of the recovery rate value will determine the extent to which the comparison of the number of CL produced by each cow with the total embryos collected. Before the flushing is carried out, the donor cow that has been programmed for superovulation will be palpated rectally first to determine the number of CL formed. If there is CL ≥ 3 , flushing will be continued, then an embryo evaluation will be carried out to determine the number of embryos produced. After that, the embryo calculation will be carried out by comparing the number of CL formed (Recovery Rate). The recovery rate values of Limousin and Simmental cattle in this study showed that the results were not significantly different ($P > 0.05$) (Table 5).

Table 5. Recovery rate

Cattle Herd	Recovery Rate	p-value
Limousin	90,00 $\pm$ 30,21	0,12
Simmental	84,21 $\pm$ 36,70	

According to Wiley (2017), the recovery rate is influenced by the flushing

technique and the placement of the Folley Gatherer during flushing. In this study, the flushing technique used was non-surgical. The flushing method used in this study is a non-surgical method by flushing the uterine cornua. The placement of the folley catheter greatly affects the success of embryo harvesting and will affect the recovery rate value (Darlian *et al.*, 2021).

Total Embryos Collected

Based on research conducted by Hardiyanto *et al.* (2016) stated that embryos obtained from Limousin and Simmental cattle that were fed with 15-20% protein content aged 3-6 years showed results that were not significantly different. Generally, the older the cow is, if it is given low-quality feed, it can cause a decrease in the total embryos produced, because young cows can produce optimally, in addition, environmental factors can also affect cows during handling and maintenance when checking reproductive status. The total number of embryos collected from Limousin and Simmental cattle in this study showed results that were not significantly different ($P > 0.05$) (Table 6).

Table 6. Total Embryos Collected

Cattle Herd	Embrio Terkoleksi	<i>p-value</i>
Limousin	8,15 ± 7,73	0,10
Simmental	6,64 ± 7,81	

The absence of a total difference in the embryos collected in the two cows may be due to the Limousin and Simmental cows having several follicles sensitive to gonadotropin

hormones that are not much different. Darlian *et al.* (2021) stated that embryo acquisition is greatly influenced by the level of the number of mature eggs ovulated and is influenced by the superovulation treatment carried out. In addition, the total number of embryos produced is also influenced by reproductive conditions, follicle population when superstimulating begins, and superovulation techniques. The follicle population when superovulation begins has a significant influence on the success of superovulation (Bó & Mapletoft, 2020), which will later affect embryo acquisition.

Proportion of Transferable Embryos

The proportion of transferable embryos is the ratio between the total number of embryos collected and the number of transferable embryos from all embryos produced. According to Darlian *et al.* (2021), the success of embryo production can be seen from the number of transfer-worthy embryos produced. According to Çizmecici & Güler (2018), embryo production can be stated to have economic value if the number of embryos suitable for transfer is ≥ 5 , and the cause of the low number of embryos suitable for transfer is suspected to be due to the hyperstimulation effect. The proportion of embryos suitable for transfer from Limousin and Simmental cattle in this study showed results that were not significantly different ($P > 0.05$) (Table 7).

Table 7. Proportion of Embryos Suitable for Transfer

Cattle Herd	Proporsi ELT	<i>p-value</i>
Limousin	34,86 ± 38,17	0,54

The absence of significant differences is thought to be due to the uniformity in the level of oocyte maturity when donor cows are in the superpopulation program. According to Setiawan *et al.* (2017), the diversity of oocyte maturity levels during superpopulation treatment can cause less than optimal embryo quality when compared to cows with normal conditions. Hyperstimulation can cause a decrease in the quality and number of embryos produced (Bó & Mapletoft, 2020). The timing of flushing can be a factor in obtaining the proportion of transferable embryos because flushing too quickly can result in a greater number of untransferable embryos being produced. The success or failure of embryo production can be measured by the number of transferable embryos obtained. According to Darlian *et al.* (2021), an embryo is said to be of good quality if the embryo has a morphology and developmental phase according to its developmental time.

Proportion of Degenerative Embryos

The proportion of degenerative embryos is an embryo that does not develop according to its phase, the number of embryos that do not develop normally will affect the high percentage of embryos that are not suitable for transfer (Subchan *et al.*, 2016). Based on the statement Setiawan *et al.* (2017), the existence of variations in embryo development in the same environment can affect the viability of the

embryo. The proportion of degenerative embryos of Limousin and Simmental cattle in this study showed results that were not significantly different ($P > 0.05$) (Table 8).

The results of this study are estimated because the induction of FSH hormone in the superovulation program stimulates the growth of the same embryo so that the quality of degenerative embryos is not significantly different in the Limousin and Simmental cattle groups. One of the benchmarks for success in the superovulation program is the high level of fertilization because it can affect the total embryo acquisition (Darlian *et al.*, 2021).

Table 8. Proporsi Embrio *Degenerative*

Cattle Herd	Degenerative	p-value
Limousin	23,70 ± 64,35	0,47
Simmental	33,80 ± 24,58	

Several factors can affect embryo production in cattle, the physiological condition of the livestock when harvesting is carried out, the level of fertilization, disturbed embryo development, and the condition of the ovaries (Hardiyanto *et al.*, 2016).

Proportion of Unfertilized Ova

The diversity in the level of oocyte maturity in superovulation treatment can cause the quality of embryos produced by donor cows to be less than optimal when compared to normal conditions. If the egg cells are not yet mature when the fertilization process occurs, it will cause no embryo formation (unfertilized), or the embryo will die (degenerative) (Setiawan *et al.*, 2017). The proportion of unfertilized

Limousin and Simmental cattle in this study showed results that were not significantly different ($P > 0.05$) (Table 9).

Table 9. Proportion *Unfertilized*

Cattle Herd	<i>Unfertilized</i>	<i>p-value</i>
Limousin	30,22 ± 39,42	0,06
Simmental	18,34 ± 31,42	

The absence of differences in the results of the study can be caused by the use of semen when the IB process is carried out on livestock. According to Hardiyanto et al. (2016)., the use of different semen can be the cause of high UF variations because each individual has a different response during the fertilization process, individual variations in cows and stud breeds can affect differences in embryo development. The acquisition of UF proportions in research can be influenced by epigenetic factors that can play an important role in the early stages of embryonic development, such as amino acids, ions, vitamins, energy substrates, hormones, growth factors, and cytokines (Hardiyanto et al., 2016). Furthermore, Setiawan et al. (2017) states that dead (degenerative) embryos or unfertilized oocytes can cause a decrease in the quality of other embryos.

CONCLUSION

There was no significant difference in the number of CLs, response rate, recovery rate, total embryos collected, the proportion of transferable embryos, the proportion of degenerative embryos, and the proportion of

unfertilized in the two cattle groups studied. However, when viewed from the average, Limousin cattle showed a better response than Simmental cattle.

ACKNOWLEDGMENTS

Thank you to the leadership and staff of the Cipelang Livestock Embryo Center, Bogor, who helped provide the three herds of cattle used in this research. And has helped the process of implementing and collecting research data.

REFERENCES

- Baharun, A., A. M.Diansyah, R. Handarini, S.Sikin, & ,P.I.Ningtias. 2025. A comparative study of intramuscular versus epidural administration of follicle-stimulating hormone for embryo production in Wagyu Cattle. *15*, 2103–2111.
- Bó, G. A., & R.J.Mapletoft. 2020. Superstimulation of ovarian follicles in cattle: gonadotropin treatment protocols and FSH profiles. *Theriogenology*, 150, 353–359.
- Çizmeçi, S. Ü., & M.Güler.2018. Superovulation in cows: A review. *International Journal Of Veterinary Science*, 7(2), 65–68.
- Darlian, F., S.Wahjuningsih, A.Rosmayanti, S.Jodiansyah, L.A.Jalaludin, Y. Setiawan, & T.Susilawati.2021. Respon superovulasi sapi persilangan Belgian Blue dengan metode yang berbeda. *Jurnal Agripet*, 21(2), 178–186.

- Djunaedi, M., R. Handarini, & D. Zamanti. 2019. Efektivitas penyuntikan FSH secara subkutan dan intramuskular terhadap respon superovulasi sapi Simental. *Jurnal Peternakan Nusantara*, 4(1), 41–50.
- Furkan, M., C. Dursun, & A. Dinc. 2023. The effect of different FSH administration before ovum pick up on superstimulation response and oocyte yield. *April*, 1055–1062.
- Hardiyanto, D., C. Sumantri, & D. Zamanti. 2016. Kualitas embrio pada sapi simmental dan limousin dengan kadar protein pakan berbeda. *Jurnal Ilmu Produksi Dan Teknologi Hasil Peternakan*, 4(2), 319–324.
- Hartati, L., T. P. Rahayu., & B. Irawan. 2022. Tingkah laku makan sapi Limousin dan Simental di Desa Ngargomulyo dan Desa Sumber, Kecamatan Dukun, Kabupaten Magelang, Jawa Tengah. *Journal of Livestock Science And Production*, 6(2), 450–461.
- Liamanu, S., L. R. Ngangi, S. H. Turangan, & J. H. Manopo. 2018. Respon ovarium sapi Limousin dan Simmental terhadap Induksi Fol. *Zootec*, 38(2), 396–406.
- Maqhashu, A., M. L. Mphaphathi, P. J. Sebei, V. Muchenje & T. L. Nedambale. 2021. Superovulation response and embryo quality recovered following flushing nguni heifers and cows. *American Journal of Animal and Veterinary Sciences*, 16(4), 272–277.
- Motta, J. C. L., C. B. Hayden, R. V. Sala, V. A. Absal, D. Pereira, J. F. Moreno, & A. García-Guerra. 2027. Synchronization of follicle wave emergence before ovarian superstimulation with fsh and ovum pick-up improves in vitro embryo production in pregnant heifers. *Theriogenology*, 188:2022.
- Revi, A., S. Solikhun, & M. Safii. 2018. Jaringan syaraf tiruan dalam memprediksi jumlah produksi daging sapi berdasarkan Provinsi. *KOMIK (Konferensi Nasional Teknologi Informasi Dan Komputer)*, 2(1), 297–304.
- Setiawan, A., E. Dihansih, & D. Zamanti. 2017. Penggunaan preparat progesteron dan hormon GnRh dalam penentuan estrus pada program superovulasi sapi Limosin. *Jurnal Pertanian*, 8(1), 7.
- Subchan, F. A., R. Handarini & S. W. Siswanti. 2016. Perbedaan Waktu Penyuntikan Hormon FSH Terhadap Respon Superovulasi Sapi Angus. *Jurnal Peternakan Nusantara*, 2(1), 159–166.
- Tjahjani, C. M. P., S. Herijanto & Y. M. Viastika. 2021. Skema optimalisasi respon transfer embrio sapi: analisis deskriptif manajemen kelompok ternak donor dan resipien. *Media Peternakan*, 22(2), 23–29.
- Wiley, C. E. 2017. Methods to enhance embryo quality and recovery rates in

superovulated beef cows. *Animal*
Physiology, 1–117.