

***Effect of Combination of Gonadotropin Releasing Hormone (GnRH) and Vitamin E-Selenium on the Success Rate of Artificial Insemination of Repeat Breeding Beef Cattle***

**Pengaruh Kombinasi Gonadotropin Releasing Hormone (GnRH) Dan Vitamin E-Selenium Terhadap Tingkat Keberhasilan Inseminasi Buatan Pada Sapi Potong Repeat Breeding.**

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### **ABSTRACT**

*This research was conducted to determine the effect of the use of Gonadotropin Releasing Hormone (GnRH) and Vitamin E-Selenium on cows experiencing reproductive disorders in the form of repeat breeding. This study used 20 repeat breeder beef cattle which were divided into 4 groups and 3 treatments and 5 replicates: P0 (control), P1 (AI+GnRH 5 ml), P2 (AI+Vitamin E-Selenium 20 ml), P3 (AI+GnRH 5 ml+Vitamin E-Selenium 20 ml). The NRR and CR data obtained were analyzed using the Cochran's Q Test, which if the test showed a real effect, the Pairwise Comparison test would be continued. The results of the study stated that there was no real effect of the combination of GnRH hormone and Vitamin E-Selenium on the success rate of Artificial Insemination in repeat breeding beef cattle ( $P>0.05$ ). In the P3 treatment, the pregnancy rate was 80% and P1 also produced the same pregnancy rate, which was 80%. This figure is higher when compared to the results of P0 which is 0% and P2 which is 40%. Based on the four groups and 3 treatments, it can be seen that the addition of a combination of GnRH and Vitamin E-Selenium injection has a higher influence in terms of the percentage of pregnancy, Non Return Rate and Conception Rate produced. This is because GnRH is able to induce a pre-ovulation LH surge. Increased LH concentrations promote oocyte maturation, induce ovulation and can improve the function of the corpus luteum. Abstracts must be written in Indonesian Language.*

**Key Words** : Beef Cattle, GnRH, Repeat Breeding, Vitamin E-Selenium

### **INTRODUCTION**

Indonesia is a potential area for the development of beef cattle due to the availability of land, abundant feed, and a suitable climate that makes the number of beef cattle spread across various regions in Indonesia (Sudarmono dan Sugeng, 2016). Beef is a food ingredient that is a supplier of protein for the domestic

community. Beef is one of the essential animal proteins needed by the human body. According to Socheh et.al., (2018) stated that currently, the need for beef in Indonesia is met through three main sources, namely from people's livestock, the livestock industry and meat imports. Beef cattle breeding activities are mostly carried out by farmers in rural areas (Rusdiana et.al., 2016). Since ancient times, cows have been domesticated for human food needs and produce milk and meat as a source of protein. Therefore, it is necessary to make efforts to increase the cattle population in the community. The technology that supports these efforts is Artificial Insemination (AI). A high success rate of Artificial Insemination is required for the efficiency of increasing the number of pregnant cows.

Meanwhile, one of the obstacles in increasing the success of artificial insemination is the existence of cases of reproductive disorders in cows. According to Rusdiana, (2019) stated that the government is improving the nursery sector to produce superior seeds and seeds in beef cattle which are the main producers of beef. One of the steps taken is to deal with the problem of reproductive disorders and save productive female cows and carry out livestock disease countermeasures as an effort to strengthen the aspect of livestock breeding. According to Marbun et.al., (2023) traditional cattle rearing is very susceptible to reproductive failure caused by inadequate seed quality and the feed provided is of poor quality. The availability of forage for animal feed with sufficient quantity and quality is one of the factors that can determine the success of businesses in the field of beef cattle, both large and small scale (Rusdiana et.al., 2016).

Cases of reproductive disorders in cows that are commonly found in the field are repeat breeding or repeated mating. Recurrent mating is a condition in which female cows experience a normal estrus cycle but when mated more than 2 times they are not pregnant (Prihatno et.al., 2021). Repeated mating in cows can cause losses to farmers because it slows down the calving interval, increases the cost of mating, and increases the number of cows that must be fed. Therefore, a solution is needed to overcome this problem. The innovation in the use of GnRH and Vitamin E-Selenium is one of the efforts to find a solution to overcome the problem of reproductive disorders. According to Perez-Marin et al., (2012) this case of repeat breeding is caused by several factors, including hormonal dysfunction, poor follicle development and anatomical abnormalities of the reproductive organs. In addition, repeat breeding is also caused by ovulation abnormalities, anovulation, ovulation delay and decreased reproductive organ function (Siregar et.al., 2022).

Gonadotropin Releasing Hormone (GnRH) stimulates the pituitary gland to produce Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) which both have the function of stimulating the ovaries to become active which is characterized by the growth of follicles and corpus luteum. Reproductive performance will take place normally if the pituitary-pituitary-gonads function optimally (Prihatno et.al., 2021). Andriani et.al., (2023) stated that Vitamin E acts as an antioxidant that can prevent the oxidation of unsaturated fatty acids in cells and can accelerate the embryogenesis process and improve reproductive outcomes. Vitamin E has a significant role in the reproductive system because of its ability to increase sperm maturity in males and increase fertility in female reproductive organs (Fauziah et.al., 2013). Meanwhile, according to Kurnia et.al., (2020) Selenium (Se) functions as an antioxidant that can prevent damage to chromosomes and maintain fertility.

Based on research conducted by Prihatno et al., (2021) it can also be known that research on the use of Gonadotropin Releasing Hormone (GnRH) to determine its effect in cases of repeated mating in cows has been carried out by several

researchers with various variations in the addition of other ingredients. However, the results are inconsistent. That is why, with the difference in the results of the study, the author is interested in conducting research on the use of GnRH combined with Vitamin E-Selenium in artificial insemination activities. With this study, it will be known whether the innovation of GnRH and Vitamin E-Selenium treatment, which is a new form of variation of research with this concept, will be better than existing research. Research on the use of GnRH in cattle undergoing repeat breeding has existed but uses a different combination such as iodine, povidone, and vitamin ADE. All of these studies produce different numbers. With the case of repeat breeding in cattle that occurs in the field, the use of the combination of GnRH and Vitamin E-Selenium is expected to increase the success of artificial insemination. The author seeks to conduct a study entitled "The Effect of the Combination of Gonadotropin Releasing Hormone (GnRH) and Vitamin E-Selenium on the Success Rate of Artificial Insemination in Repeat Breeding Beef Cows" with the hope of being able to be a solution to overcome cases of repeat breeding reproductive failure in beef cattle in community farms.

## **MATERIAL AND METHOD**

### **A. Location and Time**

This research was carried out at the Food Security and Agriculture Office of Jepara Regency, Central Java, namely on livestock owned by residents in the Kembang District, Jepara, Central Java. This research was carried out from March to June 2024.

### **B. Materials**

The materials used in this study are female beef cattle, cow straw, GnRH hormone, vitamin E-selenium, water for injection, liquid nitrogen (N<sub>2</sub>), alcohol, cotton, tissue, pregnancy test kit and soap.

### **C. Tools**

The tools that will be used in this research activity are Inseminating Gun Cow, 10 ml and 20 ml Syringes, 18G needles, Plastic Sheat, Plastic Glove AI, Containers, Short Tweezers, Scissors, Dippers and Test Tubes.

### **D. Research Variables**

#### **1. Pregnancy**

Pregnancy is the male (Spermatozoa) and female sex cells (Ovum) that fuse into a new cell known as a zygote (Widiarso, 2023). Pregnancy is data that states the success of Artificial Insemination in the form of pregnancy/non-pregnancy of cows after AI mating. The percentage of pregnancy is the number produced from pregnancy obtained after pregnancy examination (Hafizuddin et.al., 2011).

#### **2. Non Return Rate**

Non Return Rate (NRR) is the number of female cows that do not show signs of re-fertilization after 30-60 days after AI (Wiranto et.al., 2020). The NRR calculation is carried out with the following formula:

$$\text{NRR} = \frac{\text{Number of cows that do not estrus again}}{\text{Number of Acceptors}} \times 100\%$$

### 3. Conception Rate

Conception Rate (CR) is a measure of fertility statistics based on the number of pregnant cows after the first marriage (Dirgahayu et.al., 2015). The CR formula is as follows:

$$\text{NRR} = \frac{\text{Number of pregnant females in the 1st AI}}{\text{Number of Acceptors}} \times 100\%$$

## E. Research Procedure

The research activities carried out start from preparation to completion, which include:

### 1. Livestock Selection

Livestock selection is carried out before the research begins. Cows with these criteria are obtained through the information listed in the inseminator's isikhnas application which is then marked and also from the farmer's AI card. Then the cattle belonging to the farmer who have been marked are waited until they experience natural fat. It is at this time of lust that cows begin to be in AI and are given treatment. The criteria for cattle that are samples for this study are as follows:

- a. Female beef cattle
- b. Cow age over 1.5 years
- c. Have a normal estrus cycle
- d. Not pregnant
- e. Have a Body Condition Score (BCS) of 2.5 – 3
- f. Have a history of at least more than 2 times AI is not pregnant
- g. The physical condition of the cow is in good health

### 2. Artificial insemination

Artificial Insemination (Injectable Mating) activities are carried out if the sample cows that have been marked are naturally sterilized (without the help of hormone injection). The cow that will be AI is a cow that has been registered and in accordance with the above criteria and the owner of the livestock must have been notified to provide a report if the cow has been fattened. When the cow is fattened and the farmer has contacted, the cow will be given treatment.

### 3. Giving of treatment

In this research activity, 20 cows were used. There are 4 types of treatment groups, 3 of which are carried out immediately after cattle in AI. The treatment provided is:

- a. P0 As a control where the sample cattle are only in AI and are not given any treatment.
- b. P1, namely the sample cow was given 5 ml of GnRH which was injected into the cow intramuscularly at the same time after the cow was in AI.
- c. P2 is the administration of 20 ml of vitamin E-Selenium which is injected into the cow intramuscularly at the same time after AI is carried out on the cow.

- d. P3 is the administration of GnRH (5 ml) and vitamin E-Selenium (20 ml) which are injected into cows intramuscularly at the same time after AI is carried out on the cows.

#### 4. Pregnancy Screening

Pregnancy examination can be carried out as soon as possible, namely on the 18th day after the cow in AI using the Pregnancy Test Kit. According to Syaiful et.al., (2023) The Pregnancy Test Kit is a tool in the form of a chemical reagent that can be used for the early detection of pregnancy in beef cattle by using testing on cow urine. Urine collection is carried out by luring cows to urinate in the morning. The pregnancy examination was carried out by holding 3 ml of cow urine with a test tube and then given 5 drops of reagent liquid. The cow is declared pregnant if the urine turns clear and if the cow is not pregnant, the urine will turn cloudy and there is usually sediment. According to the information on this pregnancy test kit product, the accuracy of pregnancy if tested in less than 1 month after AI is 70% and 1-2 months is 90%. Therefore, pregnancy examination with this method can be carried out 18 days after AI.

### F. Trial Design

The experimental design in this study is a Complete Random Design (RAL). The design was carried out in 4 groups with 5 repetitions so that the total number of cows used was 20 cows. The determination of the number of samples and replicates is carried out based on the following federer formula:

$$t(n - 1) \geq 15$$

$$\begin{aligned}
 T & (n - 1) \geq 15 \\
 4 & (n - 1) \geq 15 \\
 4n - 4 & \geq 15 \\
 4n & \geq 15 + 4 \\
 4n & \geq 19 \\
 N & \geq \frac{19}{4} \\
 N & \geq 4,75 \rightarrow 5
 \end{aligned}$$

Information :

t = number of treatment

n = review

By calculating using this formula, it is known that the minimum number of repetitions that must be done is as many as 5 tails. Based on these results, the Experimental Design was obtained as follows:

Table 1. Experimental Design

Review	Treatment			
	P0	P1	P2	P3
1	P0U1	P1U1	P2U1	P3U1
2	P0U2	P1U2	P2U2	P3U2
3	P0U3	P1U3	P2U3	P3U3
4	P0U4	P1U4	P2U4	P3U4
5	P0U5	P1U5	P2U5	P3U5

## G. Data Analysis

The processing of Non Return Rate (NRR) and Conception Rate (CR) data in this study was carried out based on a formula and quantitative descriptive analysis to explain the results of the calculation of the formula. Non Return Rate (NRR) and Conception Rate (CR) data were also analyzed using the Cochran's Q Test, if there was a significant difference, followed by the Pairwise Comparison test. As for pregnancy, the percentage of all cows that are pregnant in the treatment will be calculated.

## RESULT AND DISSCUSSION

The results of the study on the effect of the combination of gonadotropin releasing hormone (GnRH) and vitamin E-selenium on the success rate of artificial insemination in repeat breeding beef cattle are as follows:

Table 2. Teatment Results

Variables	P0	P1	P2	P3
Pregnancy	0%	80%	40%	80%
NRR	0%	60%	40%	80%
CR	0%	80%	40%	80%

### A. Pregnancy

The success rate of Artificial Insemination can be seen based on how many cows have successfully conceived. The results of pregnancy from this study can be seen in table 3.

Tabel 3. Pregnancy Results

Review (U)	Treatment			
	P0(Control)	P1(AI+GnRH)	P2(AI+Vit E-Selenium)	P3(AI+Vit E-Selenium+GnRH)
U1	-	+	+	+
U2	-	+	-	+
U3	-	-	+	+
U4	-	+	-	-
U5	-	+	-	+
Number of Pregnancy	0	4	2	4
Percentage of Pregnancy	0%	80%	40%	80%

According to the table above, it can be seen that P0 does not produce pregnancy. While P1 (AI+GnRH) produced a pregnancy of 80% where 4 pregnant cows were pregnant. In P2 (AI+Vitamin E-Selenium) produced 2 pregnancies, which was 40%. Then in P3, namely (AI+ GnRH+Vitamin E-Selenium) produced a pregnancy of 80%, namely as many as 4 heads. From this, it can be seen that the highest pregnancy was obtained in P1 and P3, which was 80% each.

Administration of Vitamin E-Selenium can improve fertility in cows and treat several reproductive diseases (Wiedosari & Sani, 2022). According to Prasetyani et.al., (2015) the administration of Vitamin E-Selenium can improve reproductive performance and has almost the same role as GnRH, which can increase the secretion

of LH hormone. However, based on the results of pregnancy in P2 (AI + Vitamin E - Selenium), it shows a low increase in pregnancy rate, which is only 40% when compared to the control treatment which is only 0%. From this it is suspected that vitamin E-Selenium shows a low effect on a single treatment. This conjecture is taken from the comparison of the effect of the treatment of adding GnRH which tends to be higher, which is 80% and the results of the treatment of the use of Vitamin ADE + Iodin Povidon from research from Setyorini & Prihatno, (2022) which resulted in a pregnancy percentage of 50%.

Based on the data in table 3. It is known that the administration of GnRH and Vitamin E-Selenium when compared to only given GnRH is both effective for the handling of repeat breeding beef cattle. However, it can be concluded that the use of GnRH has a dominant effect. Then when this pregnancy percentage figure is compared with the research of Prihatno et al., (2021) this study produces the same figure, which is 80%. This pregnancy percentage is also lower when compared to the results of the research by Setyorini and Prihatno, (2022) which used a combination of GnRH, Vitamin ADE and Iodium Povidon, which was 100%. The difference in numbers is due to the difference in the combination of materials used.

### B. Non Return Rate

The results of NRR observation show that the treatment of adding GnRH and Vitamin E immediately after cattle in AI can be seen in the following table:

Table 4. Average Non Return Rate

Review (U)	Treatment			
	P0(Control)	P1(AI+GnRH)	P2(AI+Vit E-Selenium)	P3(AI+Vit E-Selenium+GnRH)
U1	0	1	1	1
U2	0	1	0	1
U3	0	0	1	1
U4	0	1	0	0
U5	0	0	0	1
<b>Number</b>	<b>0</b>	<b>3</b>	<b>2</b>	<b>4</b>
<b>Average</b>	<b>0,00±0,00<sup>ns</sup></b>	<b>0,60±0,55<sup>ns</sup></b>	<b>0,40±0,55<sup>ns</sup></b>	<b>0,80±0,45<sup>ns</sup></b>

Caption :  
 0 = Estrus ; 1 = No Estrus  
<sup>ns</sup>Superscripts that are different on the same line show unreal (P>0.05)  
 P0 = No treatment/control  
 P1 = Administration of 5 ml of GnRH shortly after AI in cows  
 P2 = Administration of Vitamin E-Selenium 20 ml immediately after AI in cows  
 P3 = Administration of 5 ml of GnRH and 20 ml of Vitamin E-Selenium shortly after cattle in AI

Based on the results of data analysis using the Cochran Test, s Q obtained a result of P>0.05 which means that there is no real difference from the treatment. Based on the results of the calculation of the NRR formula in the table above, it shows that NRR is obtained at P0 (Control) of 0%, the treatment of GnRH injection immediately after AI (P1) there are 3 out of 5 cows (60%), while the treatment of Vitamin E-Selenium (P2) injection was obtained from 2 out of 5 cows (40%) and 4 out of 5 cows (80%) were given GnRH and Vitamin E-Selenium injection treatment immediately after AI.

Cows that do not show symptoms of estrus are suspected of having pregnancy. From the treatments that have been carried out, there are P0 (Control) and P2 (AI+ Vitamin E-Selenium) which are considered to have a poor NRR value, namely

NRR < 50%. Meanwhile, P1 (AI+GnRH) and P3 (AI+GnRH+Vitamin E-Selenium) have good NRR values, namely NRR > 50%. This assessment is in accordance with the statement that the NRR value is considered good at the NRR figure of > 50% (Sumiyanti et.al., 2023). The NRR number is known to be out of sync with the CR number and pregnancy, especially in P1 in each of these variable results. This is because the NRR value can also be observed at 20-60 days or 60-90 days after Artificial Insemination (Susilawati, 2011). Therefore, it can be concluded that as long as before that time, pregnant cows can still experience estrus but do not ovulate.

Reproductive disorders can occur because livestock cannot adapt to the environment. This occurs because gonadotropin hormones and steroids cannot be produced perfectly (Susilawati, 2011). This opinion is related to the allegation that repeat breeding cattle samples at P0 (Control) and P2 (AI + Vitamin E-Selenium) which are control treatments and AI + Vitamin E-selenium produce low NRR and pregnancy values, namely 0% and 40% due to an unoptimal hormonal work system. These results were lower than the results of P1 (AI + GnRH) and P3 (AI + GnRH + Vitamin E-Selenium) where the samples of repeat breeding cows each received a supply of GnRH hormone with a different combination so that the performance of the reproductive organs could be improved. This is in line with the opinion of Prihatno et.al., (2021) that the addition of GnRH in cattle shortly after AI in cattle in estrus is suspected of inducing an increase in LH – pre-ovulation. Increased LH concentration promotes oocyte maturation, induces ovulation and is able to improve corpus luteum function.

### C. Conception Rate

Conception Rate data was collected through pregnancy detection using the PregnaDrop Test Kit. The Conception Rate of this study can be seen in the following table:

Table 5. Average Conception Rate

Review (U)	Treatments			
	P0(Control)	P1(AI+GnRH)	P2(AI+Vit E-Selenium)	P3(AI+Vit E-Selenium+GnRH)
U1	0	1	1	1
U2	0	1	0	1
U3	0	0	1	1
U4	0	1	0	0
U5	0	1	0	1
<b>Number</b>	<b>0</b>	<b>4</b>	<b>3</b>	<b>4</b>
<b>Average</b>	<b>0,00±0,00<sup>ns</sup></b>	<b>0,80±0,45<sup>ns</sup></b>	<b>0,40±0,55<sup>ns</sup></b>	<b>0,80±0,45<sup>ns</sup></b>

Caption : 0 = No Pregnancy ; 1 = Pregnant  
<sup>ns</sup>Superscripts that are different on the same line show unreal (P>0.05)  
P0 = No treatment/control  
P1 = Administration of 5 ml of GnRH shortly after AI in cows  
P2 = Administration of Vitamin E-Selenium 20 ml immediately after AI in cows  
P3 = Administration of 5 ml of GnRH and 20 ml of Vitamin E-Selenium shortly after cattle in AI

Based on the results of data analysis using the Cochran test, s Q showed that there was no significant difference from the treatment (P>0.05). From this study, the CR result at P0 (Control) was 0%, at P1 (AI+GnRH) it produced CR of 80%, while at P2 (AI+Vit E-Selenium) it produced CR of 40%, and at P3 (AI+GnRH+Vit E-Selenium) produced CR 80%. The ideal conception rate in a population is 60-75%, the higher the

CR number, the more fertile the cow is, while if the CR number is lower, it is considered the opposite (Febriantoro et al., 2015).

Based on this opinion, it can be seen that P0 and P2 do not have an ideal Conception Rate (CR < 60%). Meanwhile, P1 and P3 have a CR number which is included in Ideal (CR > 75%). From these figures, it can be seen that the use of GnRH hormones combined or not combined with vitamin E-Selenium gives results in the form of the same CR number, which is 80% each.

Prasetiani et al., (2015) stated that the administration of Vitamin E-Selenium can improve reproductive performance. This is because the antioxidant activity of selenium and vitamin E is able to increase mineral and nutrient intake and improve hypothalamic function so that reproductive hormones such as LH show optimal levels (Prasetiani et al., 2015). The results of this CR are in line with the results of pregnancy. The higher the CR, the higher the pregnancy rate and vice versa. Then if this CR percentage figure is compared with the research of Prihatno et al., (2021) this study produces the same figure, which is 80%. This percentage of pregnancy is also lower when compared to the results of the research by Setyorini and Prihatno, (2022) using a combination of GnRH, Vitamin ADE and Povidon Iodium, which is 100%. The difference in numbers is caused by the difference in the combination of materials used. This is in accordance with the statement of Sumaryadi and Nugroho (2019) that GnRH can increase fertility in female cows by stimulating the growth of follicles to ovulate, thereby increasing the number of corpus luteum and placental mass so that it can increase the chance of pregnancy in livestock.

Optimal LH levels in the body can improve reproductive performance, especially in cows that experience noninfectious reproductive disorders. This is because LH accelerates follicle degraaf and induces ovulation so that it can support fertilization (Prihatno et al., (2021). From the information above, it can be assumed that the use of Vitamin E-Selenium can give low results when applied to cattle and it can be known that the GnRH hormone has a dominant influence.

## **CONCLUSION**

The conclusion of the research results "The Effect of the Combination of Gonadotropin Releasing Hormone (GnRH) and Vitamin E-Selenium on the Success Rate of Artificial Insemination in Repeat Breeding Beef Cattle" is:

1. The administration of Combination GnRH and Vitamin E-Selenium given immediately after AI in cows that have undergone natural insemination on the success rate of Artificial Insemination in beef cattle does not make a significant difference due to NRR ( $P > 0.05$ ) and CR ( $P > 0.05$ ).
2. The use of a combination of GnRH and Vitamin E-Selenium given immediately after AI can result in cows getting pregnant and overcoming cases of repeat breeding. This is concluded based on the results of the percentage of pregnancy produced by P3 (AI + GnRH + Vit E-Selenium) which is 80%.

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