

THE EFFECT OF SUPEROVULATION INDUCTION USING FSH ON INCREASING FSH AND LH CONCENTRATIONS IN NEW ZEALAND WHITE RABBITS

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ABSTRACT

This study aimed to determine the concentrations of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in New Zealand White (NZW) rabbits after superovulation induction using FSH. Six female NZW rabbits and one male NZW rabbit were used in this study. Criteria for female NZW rabbits used include having previously given birth, not being pregnant, and having a body weight of 2-3 kg. The rabbits were divided into two treatment groups; the first group was injected with physiological NaCl (NZW1, n= 3), while the second group was injected with FSH (NZW2, n= 3). Injections of physiological NaCl or FSH were administered five times at 12-hour intervals and first injection was given at 20:00 p.m. In NZW1, each injection was given physiological NaCl with volumes of 0.2:0.2+0.2;0.4+0.4 mL, respectively whereas in NZW2, each injection was given FSH with doses of 4:4+4:8+8 mg, respectively. Twelve hours after the last FSH injection, NZW2 rabbits were injected with 100 IU hCG and mated with male rabbit, while NZW1 rabbits were mated without hCG administration. On days 1, 3, and 5 after mating, blood samples were collected for FSH and LH hormone examination using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The FSH concentrations on days 1, 3, and 5 between NZW1 vs NZW2 were 34.78±1.46 vs 39.75±3.18; 41.43±4.90 vs 41.42±5.14; and 44.47±1.51 vs 30.13±0.19 mIU/mL, respectively (P>0.05). The LH concentrations on days 1, 3, and 5 between NZW1 vs NZW2 were 33.78±4.01 vs 40.18±12.36; 29.34±2.24 vs 31.31±10.13; and 42.09±11.38 vs 30.57±8.87 mIU/mL, respectively (P>0.05). It is concluded that FSH administration does not affect the concentrations of FSH and LH in NZW rabbits. The FSH concentration five days after mating tends to fluctuate, while the LH concentration tends to decrease as the days increased.

Key words: FSH, LH, NZW rabbits , superovulation

ABSTRAK

Penelitian ini bertujuan mengetahui konsentrasi follice stimulating hormone (FSH) dan luteinizing hormone (LH) kelinci New Zealand White (NZW) setelah induksi superovulasi menggunakan hormon FSH. Dalam penelitian ini digunakan enam kelinci NZW betina dan satu ekor kelinci NZW jantan. Kriteria kelinci NZW betina yang digunakan meliputi sudah pernah beranak, tidak bunting, dan memiliki bobot badan 2-3 kg. Kelinci dibagi dua kelompok perlakuan, kelompok pertama diinjeksi NaCl fisiologis (NZW1, n= 3), sedangkan kelompok kedua diinjeksi FSH (NZW2, n= 3). Injeksi NaCl fisiologis atau FSH dilakukan lima kali dengan interval 12 jam dan injeksi pertama dilakukan pukul 20.00 WIB. Pada NZW1, tiap-tiap injeksi diberikan NaCl fisiologis dengan volume masing-masing adalah 0,2:0,2+0,2;0,4+0,4 mL sedangkan pada NZW2, tiap-tiap injeksi diberikan FSH dengan dosis masing-masing adalah 4:4+4:8+8 mg. Dua belas jam setelah injeksi dan FSH terakhir, kelinci pada NZW2 diinjeksi dengan 100 IU hCG dan dikawinkan dengan pejantan, sedangkan pada NZW1 dikawinkan tanpa pemberian hCG. Pada hari ke-1, 3, dan 5 setelah perkawinan, dilakukan koleksi sampel darah untuk pemeriksaan hormon FSH dan LH menggunakan metode enzyme-linked immunosorbent assay (ELISA). Konsentrasi FSH pada hari ke-1, 3, dan 5 antara NZW1 vs NZW2 masing-masing adalah 34,78±1,46 vs 39,75±3,18; 41,43±4,90 vs 41,42±5,14; dan 44,47±1,51 vs 30,13±0,19 mIU/mL (P>0,05). Konsentrasi LH pada hari ke-1, 3, dan 5 antara NZW1 vs NZW2 masing-masing adalah 33,78±4,01 vs 40,18±12,36; 29,34±2,24 vs 31,31±10,13; dan 42,09±11,38 vs 30,57±8,87 mIU/mL (P>0,05). Disimpulkan bahwa pemberian FSH tidak memengaruhi konsentrasi FSH dan LH pada kelinci NZW. Konsentrasi FSH lima hari setelah perkawinan cenderung fluktuatif sedangkan konsentrasi LH cenderung menurun sesuai peningkatan hari.

Kata kunci: FSH, LH, kelinci NZW, superovulasi

INTRODUCTION

The use of rabbits as experimental animals in superovulation activities has been reported by several researchers. Methods for inducing superovulation in rabbits have been reported using PMSG (Stheshkumar 2006), hMG (Kanayama *et al.* 1995), a combination of PMSG with hCG or GnRH (Saratsi *et al.* 2001; Dorra *et al.* 2008), a combination with PGF2 α (Marhaeniyanto and Kasthama 2007), and superovulation using bovine pituitary extract (Sayuti *et al.* 2022). Superovulation aims to induce a large number of follicles to ovulate and

produce many oocytes. The produced oocytes will undergo fertilization, resulting in many transferable embryos and achieving the desired pregnancy rate (Bó *et al.* 2002 as cited in Imron *et al.* 2016).

Superovulation using FSH has been applied to livestock. Kaiin and Tappa (2006) reported that PMSG, FSH (Ovagen), and pituitary-derived FSH (FSH-PTM) have been use in various studies to assess the effects of hormone administration on superovulation response in dairy and beef cow donor dams. Furthermore, according to Putroe (1996), FSH has several advantages, such as fewer anovulatory follicles, improved ovarian response

resulting in more ovulations, more obtainable embryos, and better embryo quality. Despite these advantages, FSH has its drawbacks, including difficulty in obtaining it in the domestic market, relatively high cost, and repeated administration leading to stress and reduced embryo quality.

Several reports on rabbit superovulation using FSH have shown variations in dosage and number of injections. Zhang *et al.* (2017) used a dose of 30 IU/rabbit given in 6 injections over 3 consecutive days. Kauffman *et al.* (1998) stated that a 4-day FSH administration in rabbit superovulation can improve the efficiency of rabbit reproductive biotechnology after embryo cryopreservation. However, Techakumphu *et al.* (2002) reported no difference in the number of ovulations and developing follicles in New Zealand White (NZW) rabbits induced with FSH doses of 21, 28, and 40 mg with 5 injections at 12-hour intervals.

Ovulation and superovulation induction in rabbits have been reported to increase FSH and LH concentrations where these increase occurs fluctuatingly. For example, FSH concentration in NZW rabbits induced using 50 IU hCG was 6.0 ± 1.6 ng/mL and then decreased 72 hours after mating (Mills *et al.* 1981). Hashimoto *et al.* (2007) reported that the peak of FSH concentration in superovulating rabbits occurred 50 hours after treatment. The LH concentration in rabbits that were superovulated using GnRH increased compared to the concentration in control rabbits. The peak concentration was reached 1 hour after treatment and then gradually decreased 3-6 hours after treatment (Cervantes *et al.* 2015). Therefore, this study aims to determine the FSH and LH hormone profiles in NZW rabbits after superovulation with a dose of 28 mg FSH administered five times.

MATERIALS AND METHODS

In this study, six female NZW rabbits with a history of parturition, weighing between 2-3 kg, not pregnant, and one male NZW rabbit were used. Prior to treatment, the rabbits were acclimatized for 30 days and housed separately (Kanayama *et al.* 1995). They were provided with specific rabbit feed 552 sp. and water *ad libitum*. After the acclimatization period, the rabbits were divided into two treatment groups: the control group (NZW1, $n=3$) injected with physiological saline solution at a total dose of 1.4 mL, and the treatment group (NZW2, $n=3$) injected with FSH hormone (Folltropin-V, Vetquinol, Australia) at a total dose of 28 mg followed by injection of hCG (Chorulon, Intervet) at a dose of 100 IU. Injections in both groups were administered intramuscularly five times at 12-hour intervals over 3 consecutive days.

Research Procedure Superovulation

Intramuscular FSH injections for superovulation in the NZW2 group were first administered at 20:00 p.m. and repeated every 12 hours until the fifth injection. Each injection of FSH, with a total dose of 28 mg per rabbit, was administered as follows: 4 mg (1st injection), 4 mg

(2nd injection), 4 mg (3rd injection), 8 mg (4th injection), and 8 mg (5th injection) (Techakumphu *et al.* 2002). Twenty-four hours after the last FSH injection, NZW2 rabbits were injected with 100 IU hCG and mated with a male rabbit. For the control group (NZW1), only physiological saline solution was injected, with a total dose of 1.4 mL per rabbit, administered as follows: 0.2 mL (1st injection), 0.2 mL (2nd injection), 0.2 mL (3rd injection), 0.4 mL (4th injection), and 0.4 mL (5th injection), followed by mating.

Blood Collection and Preparation

Blood samples were collected on days 1, 3, and 5 after mating for FSH and LH hormone analysis. Blood was collected at 08:00 a.m. through the auricular vein using a 24G IV catheter and placed in EDTA 3 mL vacutainers, and subsequently centrifuged for 15 minutes at 3500 rpm to obtain plasma samples. The separated plasma was transferred to labeled microtubes and stored in a freezer at -20°C until sample analysis process is carried out. Measurement of FSH and LH to determine hormone profiles was conducted using the Enzyme-Linked Immunosorbent Assay (ELISA) method.

Measurement of FSH and LH Concentrations

The concentration of FSH and LH was determined using the ELISA method according to the manufacturer's instructions (Bioenzy, Indonesia). The analytical sensitivity of the FSH and LH kits was 0.12 mIU/mL, with intra-assay and inter-assay coefficients of variance $< 8\%$ and $< 10\%$, respectively.

Data Analysis

The concentration data of FSH and LH hormones obtained in this study were analyzed using two-way analysis of variance (ANOVA), and the FSH and LH hormone profiles will be presented graphically.

RESULTS AND DISCUSSION

The concentration of FSH on days 1, 3, and 5 between NZW1 and NZW2 was 34.78 ± 1.46 vs 39.75 ± 3.18 ; 41.43 ± 4.90 vs 41.42 ± 5.14 ; and 44.47 ± 1.51 vs 30.13 ± 0.19 mIU/mL ($P > 0.05$) as presented in Table 1. The concentration of LH on days 1, 3, and 5 between NZW1 and NZW2 was 33.78 ± 4.01 vs 40.18 ± 12.36 ; 29.34 ± 2.24 vs 31.31 ± 10.13 ; and 42.09 ± 11.38 vs 30.57 ± 8.87 mIU/mL ($P > 0.05$) as presented in Table 2.

The FSH profile in the NZW2 group showed fluctuations (Figure 1). FSH concentration in NZW2 increased from day 1 to day 3, then decreased on day 5 after mating. The FSH concentration values on days 1, 3, and 5 after mating were 39.75 ± 3.18 , 41.42 ± 5.14 , and 30.13 ± 0.19 mIU/mL, respectively. In the NZW1 group, FSH concentration did not show fluctuations on days 1, 3, and 5 after mating (Figure 1) and tended to increase with each day, with concentrations of 34.78 ± 1.46 , 41.43 ± 4.90 , and 44.47 ± 1.51 mIU/mL, respectively.

The concentration of LH in the NZW2 group on days 1, 3, and 5 after mating showed a decrease corresponding to the increase in days (Figure 1). Additionally, LH

concentration tended to decrease with values not significantly different on days 1, 3, and 5 after mating, which were 40.18 ± 12.36 , 31.31 ± 10.13 , and 30.57 ± 8.87 mIU/mL, respectively. In the NZW1 group, LH concentration did not show fluctuations on days 1, 3, and 5 after mating (Figure 1) and tended to increase with concentrations of 33.78 ± 4.01 , 29.34 ± 2.24 , and 42.09 ± 11.38 mIU/mL, respectively.

The lower concentrations of FSH and LH in the NZW2 group may be due to the inaccuracies in the timing of FSH and LH measurements. Blood sampling for FSH and LH hormone concentration measurements in this study was carried out at 24, 72, and 120 hours after mating. At the time of measurement, it is suspected that the hormones had completed their physiological functions due to their short half-lives. A half-life of FSH is approximately 3 hours, while LH has a half-life of approximately 30 minutes (Rahmanisa 2014). Cervantes *et al.* (2015) reported that GnRH ovulation induction in NZW rabbits showed an increase in plasma LH concentration at 1 hour post-treatment, starting to decrease at 3 hours, and reaching basal levels at 6 hours post-treatment, with LH plasma concentration measurements taken at 0, 1, 2, 3, and 6-hours post-treatment. Dadashpour *et al.* (2022) reported a significant increase in serum LH concentration in NZW rabbits 30-60 minutes after gonadorelin injection, with the peak LH

secretion at 71 minutes, while for FSH measurement, an increase occurred at 50-71 hours after FSH superovulation, then gradually decreased (Hashimoto *et al.* 2007). However, Kimura (2016) reported that FSH was first detected at 2 hours, with concentrations at 12 hours, and still detectable after 3 days.

The decrease in FSH concentration can also occur due to the growth of new follicles filling the ovaries in preparation for the next cycle after the ovulation of large follicles. These newly growing follicles may release an increased amount of inhibin, which can reduce FSH secretion. An increase in FSH can occur if mating results in the release of pre-ovulatory LH and FSH, leading to ovulation of large ovarian follicles. The ovulation of these follicles leads to a decrease in circulating inhibin levels, resulting in an increase in blood FSH levels. The increase in FSH concentration occurs at 10-24 hours, peaks at 16-36 hours, and decreases 48-72 hours after mating. LH concentration increases at 2 hours and decreases at 12-72 hours after mating (Mills *et al.* 1981).

The treatment method of injections five times with a 12-hour interval in this study is suspected to affect the FSH and LH hormone profiles. According to Bó and Mapletoft (2020), twice-daily FSH injections can cause stress in donor animals, potentially reducing superovulation response. This is supported by Bova *et al.* (2014), who reported that stress can cause disruptions

Table 1. Mean ($\pm$ SD) concentration of FSH and LH in local rabbits on days 1, 3, and 5 after mating following superovulation induction with FSH.

Treatment	FSH concentration (mIU/mL) on mating day		
	1	3	5
NZW1 (control)	34.78 ± 1.46^{aA}	41.43 ± 4.90^{bA}	44.47 ± 1.51^{cA}
NZW2 (FSH)	39.75 ± 3.18^{aB}	41.42 ± 5.14^{bB}	30.13 ± 0.19^{cB}

^{a,b,c}Different superscript in the same row indicate significance difference ($P < 0.05$); ^{A,B}The same superscript in the same column indicate not significantly difference ($P > 0.05$)

Table 2. Mean ($\pm$ SD) concentration of LH (mIU/ml) in NZW1 and NZW2 rabbits on days 1, 3, and 5 after mating

Treatment	LH concentration (mIU/mL) on mating day		
	1	3	5
NZW1 (control)	33.78 ± 4.01^{aA}	29.34 ± 2.24^{bA}	42.09 ± 11.38^{cA}
NZW2 (FSH)	40.18 ± 12.36^{aB}	31.31 ± 10.13^{bB}	30.57 ± 8.87^{cB}

^{a,b,c}Different superscript in the same row indicate significance difference ($P < 0.05$); ^{A,B}The same superscript in the same column indicate not significantly difference ($P > 0.05$)

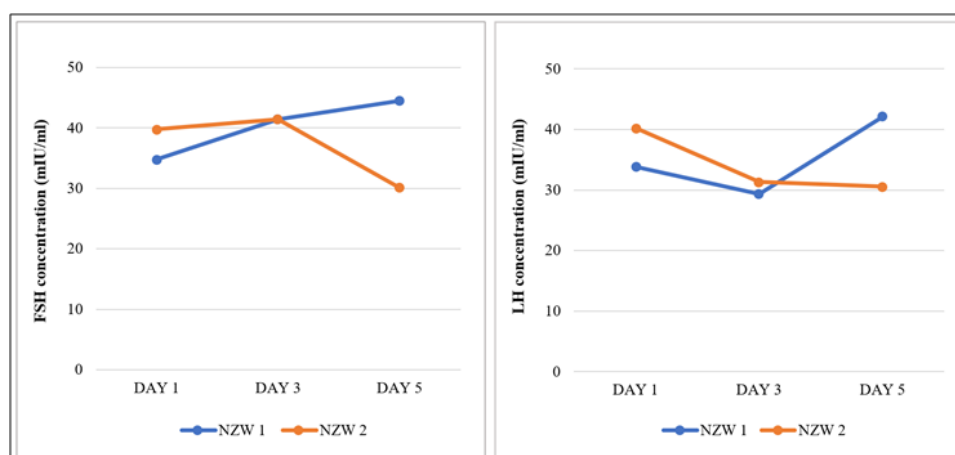


Figure 1. Concentration profile of FSH and LH in NZW1 and NZW2 rabbits (Concentrations were measured on days 1, 3, and 5 after mating)

such as inhibiting the secretion of reproductive hormones such as FSH, LH, testosterone, and estradiol.

The timing of measurements in this study was based on considerations to determine the profile of gonadotropin hormones (FSH and LH) and steroid hormones (estrogen and progesterone) and their interactions after superovulation events. Considering that the main hormones to be evaluated for superovulation success are progesterone hormones, the selection of days is based on the graduation of these hormones after superovulation. Another limitation is continuous examination with short time intervals, which is suspected to cause stress in rabbits, potentially altering their hormone profiles.

CONCLUSION

It can be concluded that the administration of FSH in NZW rabbits does not affect the FSH and LH concentration. Fluctuation of FSH concentration occurred five days after mated, while LH concentration tend to decrease as the days inceraised.

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