Research Article

Ovarian and Hormonal Responses of Local Rabbits After Superovulation Induction with Bovine Pituitary Extract

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Abstract | Superovulation using gonadotropin hormones are characterized by several limitations, leading to a wide variation in the resultant response. To overcome these limitations, several studies have recommended the use of bovine pituitary extract (BPE) as an effective alternative in improving animal reproduction. Therefore, this study aims to determine the ovarian and hormonal responses of local rabbits after superovulation induction with BPE. The sample comprised of 1 local male rabbit and 9 local female rabbits with a history of giving birth and weighing between 1.8-2.2 kg. The animals were adapted for 30 days in separate cages and provided with feed and water ad libitum before treatment. The rabbits were divided into 3 treatment groups (n=3), the first group as a control group (R1) was injected with physiological NaCl, while group two (R2) and group three (R3) were injected with FSH and BPE, respectively. The total volume of NaCl in R1 and BPE in R3 was 2.6 ml each while the total dose in R3 was 28 mg. Intramuscularly injections in both groups were performed 5 times with a 12-hour interval. The administration of NaCl in R1 and BPE in R3 was 1; 0.5+0.5; 0.3+0.3 mL, respectively while the injection of FSH in R2 was 4; 4+4; 8+8 mg. Subsequently, rabbits in R2 and R3 were injected with 100 IU hCG and mated with males, while those in R1 were mated without hCG administration 24 hours after the last FSH and BPE treatment. On the 6th day after treatment, rabbits in R1, R2, and R3 were slaughtered for ovarian organ retrieval, while blood samples for progesterone, estrogen, LH, and FSH hormone concentration examination were collected on days 1, 3, and 5 after mating. The hormone concentration was analyzed using the enzyme-linked immunoassay (ELISA) method, and the data obtained were analyzed using analysis of variance. The macroscopic observation results showed that the ovaries of rabbits in R2 and R3 were larger than those in R1. Overall, the progesterone concentration in R3 was higher compared to R1 and R2 ($P<0.05$), and the levels in R2 were higher than R1 ($P<0.05$). In addition, the number of implantation site showed a significant difference between R1 with R2 and R3 ($P<0.05$). The concentration of estrogen in R3 increased on day 5 after mating, while FSH and LH showed no significant differences between all groups on days 1, 3, and 5 after mating. Based on these findings, the administration of BPE could be considered as an alternative superovulation preparation in local rabbits in terms of ovarian and hormonal positive responses.

Keywords | Bovine pituitary extract, Hormonal, Induction, Local rabbits, Ovarian, Superovulation

Received | January 29, 2024; **Accepted** | May 24, 2024; **Published** | June 27, 2024
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Citation | Hafizuddin, Wahyuni S, Siregar TN, Sutriana A, Triyuliani R, Herviani SR, Syuhada MF, Salimah N, Eriswandi MM, Ayu RP, Nufus PH (2024). Ovarian and hormonal responses of local rabbits after superovulation induction with bovine pituitary extract. Adv. Anim. Vet. Sci., 12(8):1539-1547.
DOI | https://dx.doi.org/10.17582/journal.aavs/2024/12.8.1539.1547
ISSN (Online) | 2307-8316

INTRODUCTION

Pregnant Mare Serum Gonadotropin (PMSG) and Follicle-Stimulating Hormone (FSH) are hormones that play an essential role in superovulation induction, each offering distinct advantages and disadvantages. During experimental animal superovulation, PMSG is widely used, but its prolonged half-life often leads to residual presence in the bloodstream for several days after treatment (Wang *et al*., 2015). Several studies have shown that this extended half-life significantly influences variations in superovulation responses and the persistence of follicles within the ovaries. This condition often leads to hormonal imbalance and renders the embryos unsuitable for transfer (Afriani *et al*., 2020). Meanwhile, FSH typically induces better ovarian responses than PMSG due to its ability to produce a greater number of ovulated ova, fewer anovulatory follicles, and more high-quality embryos. Despite its advantages, a notable limitation lies in the susceptibility of FSH to adverse effects when administered in improper or excessively high doses, particularly affecting follicle development (Karl *et al*., 2021).

The administration of FSH typically comprises a twice-daily regimen spanning 3-4 days (Demoustier *et al*., 1988; Menchaca *et al*., 2007). However, this regimen can induce stress in animals and elevate the overall cost of superovulation implementation. Previous reports on rabbit superovulation using the hormone have shown several variations in terms of dose and injection frequency. For instance, Zhang *et al*. (2017) used FSH at dose of 30 IU/rabbit which was administered through 6 injections for 3 consecutive days. Kauffman *et al*. (1998) stated that FSH administration for 4 days in superovulation could enhance the efficiency of reproductive biotechnology after embryo cryopreservation. Meanwhile, Techakumphu *et al*. (2002) reported that FSH administration was performed only 5 times with a 12-hour interval.

To overcome the limitations associated with PMSG and FSH, several studies have explored alternative substances, with pituitary extract emerging as a viable option. Bovine Pituitary Extract (BPE) is a rich source of hormones, containing at least 10 bioactive substances, including FSH, Luteinizing Hormone (LH), Growth Hormone (GH), Luteotropic Hormone (LTH), Adrenal Corticotropic Hormone (ACTH), Thyroid Somatotropic Hormone (TSH), and Lipogenic hormone, all produced by the anterior pituitary lobe. Recent reports have shown the potential of pituitary extract in inducing estrus in dairy cows and enhancing reproductive efficiency in goats (Siregar *et al*., 2013). Kaligesing goats injected with pituitary extract intramuscularly exhibited early estrus behaviors, suggesting its potential to improve estrus behavior performance in Etawah goats (Setiawan *et al*., 2019). Moreover, Hafizuddin *et al*. (2010) showed that it had similar effectiveness as PMSG in inducing superovulation in mice. The use of pituitary extract has been reported to present several advantages over synthetic hormones due to its ability to produce gonadotropins, such as FSH, LH, and GH, which play a role in fetal growth and maternal milk production. The natural gonadotropins and GH in pituitary extract do exert negative impacts on the reproductive performance of female donor animals (Nalley *et al*., 2017).

The use of pituitary extract for superovulation purposes remains limited (Arum *et al*., 2013; Sayuti *et al*., 2022), with precise methods and doses yet to be firmly established. In a study by Sayuti *et al*. (2022), BPE was administered to rabbits once daily for 3 days, with decreasing doses of 1, 0.5, and 0.3 ml. The results showed that the number of fetuses as well as the concentration of estrogen and progesterone showed no significant differences compared to the control group. The study also suggested that BPE administration tends to increase progesterone concentration in local rabbits, showing a hormonal increase compared to the control. On day 7 after mating, the progesterone levels in BPE-injected and control groups were 10.00±5.30 and 3.72±1.04 ng/mL, while the values on day 14 were 11.95±5.38 and 1.01±0.65 ng/ml, respectively. The progesterone increase can be attributed to the effect of the increased ovulation caused by BPE. Siregar *et al*. (2020) also reported that BPE administration in white rats could enhance folliculogenesis activity, as showed by an increase in the number of follicles and estrogen concentration. Based on these findings, it was presumed that its ability in superovulation induction was similar to FSH. Therefore, treatment protocols must follow the FSH administration pattern.

The success of superovulation can be showed by an increased number of offspring (Nur *et al*., 2016), the concentration of estrogen and progesterone hormones (Amiruddin *et al*., 2013), and an increase in the number of corpus luteum (CL) (Siregar *et al*., 2020). Several studies have shown that ovulation and superovulation induction responses in rabbits can increase FSH and LH levels. FSH concentration has been reported to fluctuate more than LH after induction. In New Zealand White (NZW) rabbits induced ovulation with 50 µg hCG, the concentration of the hormone was 6.0±1.6 ng/ml and then decreased to pre-mating levels 72 hours after mating (Mills *et al*., 1981). Hashimoto *et al*. (2007) reported that the FSH peak concentration in rabbits super ovulated occurred 50 hours after the start of treatment. LH levels in GnRH-super ovulated rabbits increased significantly compared to the controls. Furthermore, the concentration peaked at 24 hours after treatment and then gradually decreased until day 6 (Cervantes *et al*., 2015). This study aims to determine the ovarian and hormonal responses of local rabbits after

superovulation induction with BPE.

MATERIALS AND METHODS

This study was conducted after approval from the ethics committee of Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia with certificate No. 139/KEPH/VII/2020. A total of 9 local female rabbits that had previously given birth with a weight of 2-3 kg, and 1 local male rabbit were used in this study. The animals were adapted for 30 days in separate cages (Kanayama *et al*., 1995) and provided with feed and water ad libitum. Subsequently, the rabbits were divided into 3 treatment groups (n=3). The rabbit in group 1 (R1) was injected with 2.6 mL physiological NaCl, while rabbit in group 2 (R2) and 3 (R3) were injected with 28 mg FSH and 2.6 mL BPE. The intramuscularly injections were performed 5 times with a 12-hour interval. The preparation of BPE was carried out based on the method by Isnaini *et al*. (1999).

Superovulation

The initial injection of physiological NaCl in groups R1, FSH in R2, and BPE in R3 was conducted at 08:00 PM and repeated every 12 hours until the 5th injection. In group R2, each injection comprised FSH with doses of 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). In group R3, each injection consisted of BPE with doses of 1 ml (1st injection), 0.5 ml (2nd injection), 0.5 ml (3rd injection), 0.3 ml (4th injection), and 0.3 ml (5th injection) (Sayuti *et al*., 2022). Rabbits in R2 and R3 were then injected with 100 IU hCG and mated with males, while those in R1 were mated without hCG injection, 24 hours after the last FSH and BPE injections.

Ovarian collection

On day 6 after treatment, rabbits in R1, R2, and R3 were slaughtered for ovarian organ retrieval. Dexter et sinister ovaries were then prepared for macroscopic morphological observations. Morphological observations focused on the superficial part to identify follicle and CL types, as well as to count the number of follicles and CL in the dexter et sinister ovaries.

Blood sampling and hormonal determination

Blood samples for examining the concentration of progesterone, estrogen, LH, and FSH were collected on days 1, 3, and 5 after mating at the same time. Rabbit blood was collected using a 1 ml disposable syringe through the auricular vein. The blood was collected in a 3 ml EDTA vacutainer and centrifuged for 15 minutes at 3500 rpm. The plasma was then transferred to coded microtubes and stored in a -20 °C freezer until examination.

Hormone concentration analysis was performed using ELISA method. The measurement procedures for progesterone and estrogen concentration followed the ELISA kit manual (DRG, International Inc., Germany), while the concentration of FSH and LH was determined using the ELISA kit manual (Bioenzy, Indonesia). The P4 kit analytical sensitivity was 0.045 ng/mL and the variance of coefficients for intra-assay and inter-assay were ≤ 5.43% and ≤ 4.3%, respectively. For the E2 kit, the limit of detection was 9.714 pg/mL, while the intra-assay and inter-assay were ≤ 2.71% and ≤ 6.72%, respectively. The FSH and LH kit analytical sensitivity was 0.12 mIU/mL, while the variance of coefficients for intra-assay and inter-assay were < 8% and < 10%, respectively.

Data analysis

The data obtained in this study were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's test.

RESULTS AND DISCUSSION

Macroscopic observations of ovarian responses in local rabbits

Macroscopic observations of ovarian responses in the 3 rabbit groups focused on the changes in the superficial part of the ovaries, including follicles at various stages and CL. Based on the observations on the rabbits ovaries in R1, R2, and R3 showed differences in morphology as presented in Figure 1.

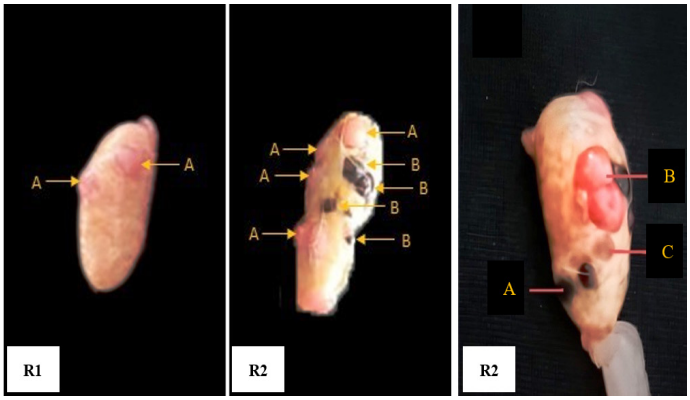

Figure 1: Morphology of the ovaries in local female rabbits (R1, physiological NaCl; R2, FSH; R3, Bovine pituitary extract; A, corpus luteum; B, corpus haemorrhagicum; C, follicle).

Macroscopically, the ovaries of rabbits in R2 and R3 were larger compared to R1. This size difference was attributed to the abundance of follicles and CL induced by superovulation with FSH and BPE. The formed follicles included primary, secondary, and tertiary (antral) variants, with CL and corpus haemorrhagicum (CH) observed among them. The macroscopic calculation results for the

number of follicles and CL on the surface of the dexter et sinister ovaries in both groups are presented in Table 1.

Table 1: The number of follicles and CL observed macroscopically in local rabbits induced with physiological NaCl and BPE.

Measurement parameters	Treatment		
	R1 (Physiological NaCl)	R2 (FSH)	R3 (BPE)
Number of follicles	13.00±1.00[a]	48.23±19.54[b]	44.33±28.57[b]
Number of CL	6.00±5.29[a]	18.7±7.8[b]	21.00±3.00[b]
Number of implantation site	3.21±1.34[a]	11.7±4.7[b]	12.3±3.5[b]

[a,b] Different superscript letters in the same row showed significant differences (P < 0.05).

The number of implantation site showed a significant difference in groups R1, R2 and R3 (P<0.05). This was consistent with the significant differences in the number of CL, as presented in Table 1. The decrease in the number of implantation site was also consistent with the decrease in ovulation. Several studies showed that this could be due to the failure of fertilization in the ovulated oocytes. Satheskumar (2006) reported a decrease in the number of fertilized oocytes compared to the number of ovulations. In NZW rabbits, the number of ovulations was 22.0±1.4, while the number of fertilized oocytes was only 6.8±3.1.

Based on the data in Table 2, there was a significant difference (P<0.05) in progesterone concentration among R1, R2, and R3. The highest progesterone concentration on days 1, 3, and 5 was found in R3. Furthermore, the estrogen level in R1 and R2 tended to remain stable, while that of R3 tended to increase by days, as shown in Table 2.

Table 2 showed that the estrogen levels in R1 and R2 tended to be lower R3 (BPE injection) on days 1 and 3 after mating, even not significantly different (P>0.05). However, estrogen levels in group R1 and R2 on day 5 exhibited a significant difference (P<0.05) compared to R3. The levels of FSH and LH which were measured in all groups on days 1, 3, and 5 after mating did not differ significantly (P > 0.05).

Ovarian responses

The results showed that superovulation induction using BPE could enhance ovarian follicle development in R2 and R3. Furthermore, the macroscopically observed follicles included small (secondary) and large (tertiary or antral) ovarian follicles that had not undergone ovulation. This finding was consistent with O'Callaghan *et al.* (2000) that superovulation induction with FSH in sheep increased the number of follicles compared to the control, with values of 12.3±1.4 and 3.5±0.3, respectively. The number of CL in R2 was higher than in R1, and this result was consistent with Ju *et al.* (2001) and Marhaeniyanto and Khastama (2007) who obtained similar results.

Ju *et al.* (2001) reported an increase in the average number of ovulations in rabbits injected with FSH superovulation compared to the control, with respective values of 37.2±16.1 and 10.4±2.6. Marhaeniyanto and Khastama (2007) also showed an increase in the number of CL in rabbits injected with a combination of PMSG and PGF2α compared to the control, with respective values of 9.00±1.15 and 6.25±1.95.

Table 2: The progesterone, estrogen, FSH, and LH concentration of local rabbits on days 1, 3, and 5 after mating and superovulation induction with BPE.

Parameters	Treatments	Hormone concentration on the day after mating		
		1	3	5
Progesterone (ng/mL)	R1 (physiological NaCl)	0.56±0.21[a]	2.83±0.99[a]	2.25±0.24[a]
	R2 (FSH)	3.63±0.83[b]	8.49±5.60[b]	18.23±14.01[b]
	R3 (BPE)	5.90±2.11[c]	13.15±2.37[c]	36.66±3.89[c]
Estrogen (pg/mL)	R1 (physiological NaCl)	18.80±4.36[a]	18.00±4.19[a]	23.53±5.09[a]
	R2 (FSH)	22.71±1.47[a]	21.59 ± 2.84[a]	24.19±4.90[a]
	R3 (BPE)	30.25±7.85[a]	38.45±26.45[a]	151.47±47.77[b]
FSH (mIU/mL)	R1 (physiological NaCl)	40.66±5.29	45.73±8.12[a]	43.52±5.22[a]
	R2 (FSH)	38.89±3.96	42.01± 6.79	40.99±4.83
	R3 (BPE)	36.64±5.46[a]	35.87±10.33[a]	25.29±12.46[a]
LH (mIU/mL)	R1 (physiological NaCl)	40.27±9.74[a]	40.54±6.13[a]	35.95±13.56[a]
	R2 (FSH)	33.06±5.55	46.40±12.09	36.54±3.30
	R3 (BPE)	32.73±7.81[a]	35.63±7.20[a]	22.20±10.76[a]

BPE: bovine pituitary extract. [a,b] Different superscript letters in the same column indicated significant differences (P < 0.05).

Hormonal response

The progesterone concentration in R2 and R3 showed that the rabbits had successfully became pregnant. Szendro *et al.* (2010) also reported that the progesterone concentration in pregnant rabbits was 9.4 ng/ml, while Kleden and Soetatto (2017) showed lower levels at 3.1 ng/ml. In New Zealand white rabbits, levels on days 7, 14, and 21 of pregnancy was 3.16±0.3, 4.00±0.32, and 3.94±0.32 ng/ml, respectively (Ashour and Abdel-Rahman, 2019). In R1, there was also a suspected pregnancy due to the tendency for an increase in progesterone concentration on day 3. Syafruddin *et al.* (2022) reported that the hormone levels in local pregnant rabbits on day 4 after mating was 2.10±1.41 ng/ml.

During the examination period, it was evident that the progesterone concentration in R3 was higher compared to R1 and R2 ($P<0.05$) and the levels in R2 was higher than R1 ($P<0.05$), primarily due to the increased number of ovulations. The formation of a higher number of CL led to an increased secretion of the hormone (Sudjatmogo *et al.*, 2001; Adriani *et al.*, 2004). Maertens and Luzi (1995) stated that superovulation caused an increase in the number of ovulating follicles. The rupture of follicles during ovulation formed a CH, which later became the CL. Furthermore, the CL was known as a temporary endocrine organ that predominantly produced progesterone. The levels of this hormone in blood serum were often used to predict the number of CL in domestic animals during the luteal phase (Scaramuzzi *et al.*, 1993). Tjitosumirat (2009) reported that progesterone concentration could be used to predict the number of ovulations in small ruminant animals.

Based on Table 2, there was an increase in progesterone concentration in R2 and R3 over the examination period. The increase in progesterone concentration with time was also reported by Sayuti *et al.* (2022), who used BPE preparation for superovulation induction. The progesterone concentration on days 7 and 14 after mating was 10.00±5.30 and 11.95±5.38 ng/ml, respectively. Challis *et al.* (1973) reported a similar trend where the average levels during pregnancy increased from 5.3 ng/ml on day 3 to 17-19 ng/ml on days 12 to 15.

A relatively high increase in hormone concentration occurred on day 5, due to progression into the functional luteal phase, where the CL began to actively secrete progesterone in large quantities. Wijono and Didi (1998) stated that high levels of progesterone occurred in the pregnancy phase with maximum CL development. The lower progesterone concentration in R1 was believed to be related to the lower number of ovulations compared to R2 and R3. The absence of a linear increase in the hormone's level in R1 could be related to the regression of several CL after mating, which did not develop optimally. Arimbawa *et al.* (2012) stated that progesterone levels increased and decreased in line with CL development during the estrous cycle. Another possibility was that the mating was unsuccessful and only led to pseudo-pregnancy. Browning *et al.* (1980) stated that progesterone concentration in local pseudo-pregnant rabbits was lower compared to pregnant rabbits. However, Holt *et al.* (1976) stated that progesterone concentration in pregnant and pseudo-pregnant rabbits did not differ during the implantation period.

The increase in progesterone concentration in R2 and R3 showed that the administration of FSH and BPE as superovulation induction had an effect on increasing ovulation in the samples. The ability of BPE to induce folliculogenesis in R3, leading to the formation of numerous CL compared to R2, suggested that BPE was superior to FSH. This finding was in line with Isnaini *et al.* (1999) that BPE contained FSH hormone and could be used as a superovulation agent. Several studies also showed that PMSG and BPE had equal effectiveness in inducing twin birth in goats (Siregar *et al.*, 2013; Zulkarnain *et al.*, 2015). According to Sayuti *et al.* (2022), BPE administration in local rabbits led to a non-significant increase in progesterone concentration compared to the control on day 7 after mating at 10.00±5.30 and 3.72±4.04 ng/ml, respectively, while this current study obtained significant results. The method of administering BPE in this study was considered to be more appropriate compared to that of Sayuti *et al.* (2022). Possible factors affecting the higher progesterone concentration compared to previous reports included the injection method performed twice a day, the amount of gonadotropin hormone present in BPE, the good physiological condition of the experimental animals, and the addition of hCG, which played a role in controlling ovulation.

Based on Table 1, the estrogen levels in R3 increased on day 5 after mating. This was consistent with Suartini *et al.* (2013) who observed that repeated doses released gonadotropin hormones, leading to an increased number of growing follicles. Ashour and Abdel-Rahman (2019) reported that estrogen concentration in NZW rabbits tended to increase with advancing pregnancy age. The levels observed on days 14, 21, and 28 of pregnancy were 47.31±4.99, 56.33±5.34, and 88.99±5.34 pg/ml, respectively. According to Siregar *et al.* (2020), the increase in the hormone's level was due to the growth of follicles.

The possible increase in estrogen on day 5 in R3 could also be due to the administration of hCG. In addition, human Chorionic Gonadotropin (hCG) had a biological activity similar to LH. Mattioli *et al.* (2021) as cited by Mattioli *et al.* (2021), stated that LH could induce earlier ovulation and increase estrogen secretion. The peak increase in the hormone coincided with the wave of follicle growth (Fortune *et al.*, 1991). The occurrence of increased growth of ovulating

follicles often led to significant production of estrogen due to the effect of PMSG administration (Maertens and Luzi, 1995). This was consistent with Sugiyatno *et al.* (2001) that the number of ovulating follicles increased the amount of the hormone in the blood serum.

In R3, there was a wide standard deviation that was suspected to affect the non-significance of the statistical test results for estrogen concentration on days 1, 3, and 5. This wide standard deviation showed a large variation among individuals. A possible cause of this observation was that rabbits in R3 came from different (proliferation) strains. In sheep, it had been reported that the effectiveness of PMSG administration was dependent on the fertility level of the strain and the treatment had no impact when given to high-fertility strains (Langford *et al.*, 1983). Lehloenya and Greyling (2010) reported that the age and parity of Boer goats affected ovarian responses after superovulation. Song *et al.* (2012) also reported that the superovulation responses in Hanwoo cows were determined by their parity. The superovulation responses, marked by a higher embryo yield, were observed more in cows with parity 3-5 compared to parity 1-2 and parity >6. Based on these findings, parity had an effect on the responses to gonadotropin, as there was a difference in ovarian sensitivity to gonadotropin induction at different ages. In this study, the sample criteria used included animals that had previously given birth, without limiting the number of calving periods. The limited number of samples was also suspected to affect the non-significant results.

Sumiyoshi *et al.* (2014) reported that estrogen levels played a crucial role in endocrine control. Furthermore, Lestari and Ismudiono (2014) stated that an increase in estrogen had both positive and negative feedback effects on the hypothalamus, leading to the release of FSH and LH. According to Fortune (1993), the processes of follicle growth, ovulation, and CL formation were affected by the circulation of reproductive hormones in the body. The hypothalamus produced GnRH, which stimulated the anterior pituitary to release FSH and LH in response to estrogen. This suggested that the significant increase in estrogen was likely caused by a positive feedback effect on the hypothalamus in producing GnRH.

The non-significant differences in FSH and LH concentration between R1, R2, and R3 on days 1, 3, and 5 after mating were likely due to inaccuracies in the time of hormone concentration measurements. Blood sampling in this study was performed on days 1, 3, and 5 after mating and it was suspected that FSH and LH concentrations returned to their baseline levels. Mills *et al.* (1981) reported that FSH levels in NZW rabbits induced with 50 IU hCG peaked between 16-36 hours after mating and returned to baseline levels 72 hours after mating. Osteen and Mills (1979) also showed that the first peak increase in its concentration occurred 2-3 hours after mating, and the second was observed at 24-48 hours. Hashimoto *et al.* (2007) stated that the peak increase in rabbits super ovulated with FSH occurred 50 hours after the initiation of treatment, while LH concentration, as reported by Osteen and Mills (1979), peaked 2 hours after mating and returned to estrus levels within 12 hours. According to Argente (2021), the highest LH levels were observed 2 hours after natural mating, which was 5 times higher compared to the value at 48 hours before mating, and gradually decreased each day afterward. Furthermore, Cervantes *et al.* (2015) showed that LH concentration was peaked 1 hour after treatment and then gradually decreased after 3-6 hours. The measurement of FSH and LH concentration in this study was conducted on days 1, 3, and 5, at the point where both hormones were likely to return to their baseline levels, leading to non-significant differences between the 3 treatment groups.

The LH concentration in R1 was relatively stable, while fluctuations were observed in R2 and R3. Furthermore, statistical analysis of LH levels showed non-significant differences ($P>0.05$). The non-significant differences in FSH and LH concentration could be attributed to the growth of new follicles in the ovaries (Osteen and Mills, 1980) in preparation for the next cycle after the ovulation of de Graaf follicles. These new follicles released inhibin, which could reduce FSH secretion (Mills *et al.*, 1981). The condition could also be due to the repetition and inappropriate interval of BPE injections. In this study, rabbit injections were repeated only 5 times over 3 days, in the morning and evening. Sayuti *et al.* (2022) stated that non-significant differences in hormone levels were often caused by the repetition factor in treatment and the interval of BPE injections. Similarly, Siregar and Hamdan (2007) mentioned that one factor to be considered for achieving optimal superovulation was repetition in treatment.

Another factor that contributed to the low levels of FSH and LH concentration was the low levels of specific hormones contained in BPE used for superovulation induction in the experimental rabbits (Sayuti *et al.*, 2022). Solihati (2006), stated that in addition to being affected by hormone levels in the blood, hormone activity also depended on the presence of receptors on target cells and transport proteins in the blood. The selection of the age of experimental animals could affect superovulation response, and this was consistent with Zhang *et al.* (2017).

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the administration of BPE could be considered as an alternative superovulation preparation in

local rabbits based on ovarian and hormonal responses.

ACKNOWLEDGEMENT

This research was funded by Universitas Syiah Kuala through 'Penelitian Profesor' for Fiscal Year 2022, in accordance with Letter of Agreement for Assignment for the Implementation of Number: 141/UN11/SPK/PNBP/2022. The author would also acknowledge the Dean of Faculty of Veterinary Medicine, Universitas Syiah Kuala, as well as the Head and Management Team of Experimental Animals Unit, which has permitted to use of research facilities.

NOVELTY STATEMENT

The use of BPE for superovulation preparation as an alternative to PMSG and FSH.

AUTHOR'S CONTRIBUTION

H and SW wrote the initial manuscript, performed manuscript revision, and data analysis. RT, SHH, MFS, NS, MM, E, RPA, and PHN participated in performing, selecting samples, sample collection, and performed practical experiments. TNS and AS developed the original idea and protocol, conducted the research, and revised the final manuscript

Conflict of interest

The authors have declared no conflict of interest.

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