Effect of Various Doses of FSH (Follicle Stimulating Hormone) on the Superovulation Response of Swamp Buffalo (B. Bubalis Carabauesis) in West Sumatera, Indonesia

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Abstract: This study aimed to determine the effect of various doses of FSH on the superovulation response of swamp buffalo. The experimental subjects used were 16 swamp buffaloes with no pregnant status, 3-5 years of age, a calf, normal estrous cycles, and clinically healthy reproduction. The swamp buffaloes were made to have synchronized estrous with double dose injection of FSH and PMSG hormones and were injected intramuscularly using four FSH doses as a treatment: 320 mg/16 ml, 360 mg/18 ml, 400 mg/20 ml, and 440 mg/22 ml. The experimental design used was CRD (Completely Randomized Design), which consisted of four replications. Parameters observed included: superovulation response, number of corpus luteum, onset, and estrous in swamp buffalo. The results obtained were superovulation response of up to 56.25% of the swamp buffaloes. The average number of corpus luteum, onset, and estrous in the swamp buffaloes were 2.75, 27.75, and 32.50 h, respectively. It was concluded that superovulation with doses of 320 mg/16 ml, 360 mg/18 ml, 400 mg/20 ml, and 440 mg/22 ml FSH showed positive responses, but 320 mg/16 ml as an optimum dose produced more corpus luteum.

Keywords: Corpus Luteum, Estrous, FSH Doses, Swamp Buffalo, Superovulation Response.

1. INTRODUCTION

Buffaloes (Bubalus bubalis) is a type of large ruminant which have a special ability to digest low-quality food to sustain life. In West Sumatra, Indonesia, the population of buffalo in 2017 was 110,236 (Statistik, 2017). In general, buffaloes raised in West Sumatra are the buffalo species called swamp buffalo (B. bubalis carabauesis). However, the productivity of swamp buffalo is low and significantly ham¬pered by various reproductive factors, namely late attain¬ment of puberty, poor estrus expression, seasonality, and post-partum anestrus (Singh et al., 2015; Safari et al., 2018; Baruselli et al., 2020; Gimenes et al., 2015). Meanwhile, livestock experiencing a silent heat is also a factor in the low productivity of swamp buffalo, such as the difficulty in making proper detection of estrous. This difficulty causes inaccurate insemination and, ultimately, fertilization failure (Chaiklun et al., 2012; De Rensis & Lopez-Gatius, 2007; Barile, 2005).

Various reproductive technologies have been applied in Indonesia, such as artificial insemination (AI), embryo transfer (ET), sperm sexing, synchronization of estrous, superovulation, embryo sexing, and cloning (Afriani et al. 2018; Madan et al., 1996; Perera, 2011). Embryo transfer (ET) is a reproductive technology in a breeding program whereby female parent seeds and superior males are utilized to increase livestock productivity (Afriani, 2015; Hasler, 1992; Pedersen et al., 2012). Programs that can help in the process of ET are superovulation programs that can produce more than two eggs in one ovulation event.

The superovulation success factor is influenced by the superovulation response to the donor parents, fertilization factor and embryo viability, the hormone injection factor used, and the donor parent management factor (Rahman et al. 2014; Velazquez, 2011). Various studies on the effect of hormone doses given for superovulation responses to donor parents had been carried out by using PMSG, FSH, Oven, GnRH, and PGF2α (Redhead et al., 2017). There are two types of hormones used in superovulation: Pregnant More Serum Gonadotrophin (PMSG) and Follicle Stimulating Hormone (FSH). However, the hormone commonly used to induce superovulation in swamp buffalo is Follicle Stimulating Hormone (FSH) obtained from the pituitary.

The superovulation response could be affected by the accuracy of selecting the time of FSH injection during follicular waves and the hormonal preparations used to induce superovulation. The use of FSH in superovulation programs could stimulate follicle growth and increase the number of mature follicles in the ovary (Singh et al., 2015). FSH is a glycoprotein hormone that has a short half-life, thus requiring repeated administration to stimulate follicular activity (Arum et al. 2013). A follicular wave occurs in the middle of estrous cycles in the mid-luteal phase, between the 9th and 12th. For buffaloes, the length of the estrous cycle range from 20-22 days. Implementation of a superovulation program will be good if done between the 9-12 days (Bearden et al. 2004). Several doses of FSH is vital for the success of superovulation. This study was conducted to determine the effect of FSH doses on the superovulation responses of parent swamp buffalo used as embryo donors.

2. METHOD

Donor Selection

In this study, 16 swamp buffaloes were used with non-pregnant status, at least two months post-partum, 3-5 years old, multiparous, healthy reproduction, and clinically normal, with normal estrous cycle (Sianturi et al. 2010).

Superovulation

Examination of the reproductive organs and corpus luteum (CL) by rectal palpation and estrous detection was done visually by observing the symptoms and estrous behavior of the swamp buffaloes synchronized with double injection using 5 ml of PGF2α hormone intramuscularly. FSH injection was started on the 10th day, and its administration was done twice a day, morning and evening (12 h interval), with decreased doses for three days intramuscularly. The dosage given was 320 mg/16 ml, 360 mg/18 ml, 400 mg/20 ml, 440 mg/22 ml.

Estrous detection

The estrous detection was calculated by the scoring system and through visual observation. The estrous symptoms and behavior observation included restlessness and irritation of the buffaloes, and the vulva became slightly oedematous with a variable amount of mucoid discharge. Furthermore, the vulva became slightly swollen and congested, and there was a slight elevation of temperature. The trail became slightly raised with the tail-head hair often ruffled, and the skin sometimes became excoriated. Decreased appetite and increased vaginal temperature were also observed (Ismaya, 2014).

Artificial insemination

Each donor was placed in a clamp enclosure, and the implementation of AI was performed 12 h after the donor experienced estrous (Carvalho et al., 2013). The AI was carried out 48−56 h after the estrous sign was observed. The implementation of AI was conducted by an insemination expert (Rabidas et al., 2017).

Parameters measured

Parameter measured includes Superovulation response, the number of corpus luteum in buffaloes were measure according to FSH dose treatments. The number of corpus luteum contained in the right ovary and left ovary were recorded. Swamp buffalo onset and estrous duration were calculated, starting from PGF2α hormone injections until estrous onset.

Statistical analysis

All data were analyzed by analyzing variance (ANOVA) of the CRD (completely randomized design) using a general linear model procedure on SPSS software version 20.0.

3. RESULT AND DISCUSSION

Superovulation response

This study shows that donor buffalo superovulated with different FSH doses responded at the rate of 56.25%. Out of the 16 swamp buffaloes given superovulation treatment, 9 donor buffaloes gave positive responses, while 7 donor buffaloes did not show any response to superovulation treatment (Table. 1).

Tabel 1. Percentage of Donor Responses to Superovulation

FSH Doses (mg)	Donor	Donor Response	Donor not Responding	Response Rate (%)
320 mg/ 16 ml	4	4	0	100
360 mg/ 18 ml	4	1	3	25
400 mg/ 20 ml	4	2	2	50
440 mg/ 22 ml	4	2	2	50
Total	16	9	7	56.25

Different doses of FSH administered to swamp buffaloes gave different responses as regards superovulation. FSH dose administration of 320 mg/16 ml gave the best result in superovulation response, with 100% of four buffalo donors producing more than one CL. The best FSH dose for swamp buffalo is therefore 320 mg/16 ml. The success of superovulation in donor buffalo could be seen from the number of CL produced in the ovary. This result is in agreement with Jordiansyah et al. (2013), who reported that an increase in the number of CL in the ovary after administration of superovulation hormone gives an idea of the success of superovulation because the superovulation goal is to produce eggs in large quantities that exceed an animal's natural ability.

Several factors also affect the success of superovulation, such as age, body condition, nutrition obtained, hormonal preparations used, and reproductive status of the donor buffalo (Bearden et al. 2004; Nilchuen et al. 2011). An increase in donors' age reduces the superovulation response due to a decrease in reproductive activity as the animals grow older. Also, malnutrition of donor animals with matured reproductive organs can reduce the swamp buffalo's superovulation response (Suardi, 2012).

The use of FSH in the superovulation program serves to stimulate follicular growth and increase the number of mature follicles in the ovary (Sakaguchi et al., 2019). The target organ of FSH is the ovary, in which the follicle contains an egg cell. The use of various doses of

FSH should be done during follicular waves. This is according to Setiadi et al. (2005), who stated that the administration of superovulation hormones at the time of follicular waves could improve superovulation response. Varying ovarian response to superovulation treatment in buffalo donors is related to diversity in ovarian follicle development during treatment.

The use of hormones at the right time, dosage, and type of hormones will affect the donor's response and embryo acquisition (Jordiansyah et al., 2013). According to Silva et al. (2009), this is, who stated that the administration of FSH at the right time would produce maximum results. Efficient use of time, energy, cost, the use of donor, and the quality and purity of FSH preparations that are used can cause differences in each animal's response. FSH dose administration of 320 mg/16 ml is the best dose for buffalo's superovulation (Feradis, 2010).

Number of corpus luteum (CL)

In buffaloes, the presence of CL can be examined by rectal palpation (Ismaya, 2014). The average number of CL formed in the swamp buffalo for a dose of 320 mg/16 ml was 2.75, for a dose of 360 mg/18 ml, the average number of CL formed was 1.25, a dose of 400 mg/20 ml gave 2, and a dose of 440 mg/22 ml gave 1.75. Statistical analysis of the data obtained shows that administering FSH at doses of 320, 360, 400, and 440 mg gave results that were not significantly different for the acquisition of CL in swamp buffalo (P> 0.05). However, the highest number of CL (11 CL) was obtained with a dose of 320 mg/16 ml FSH, and the lowest number of CL (5 CL) was obtained with a dose of 360 mg/18 ml FSH. This can be influenced by several factors, including genetic factors, nutrient content in the feed, maintenance, management, buffalo age, FSH dose, the accuracy of AI time, and application of the hormone FSH (Bearden et al. 2004)

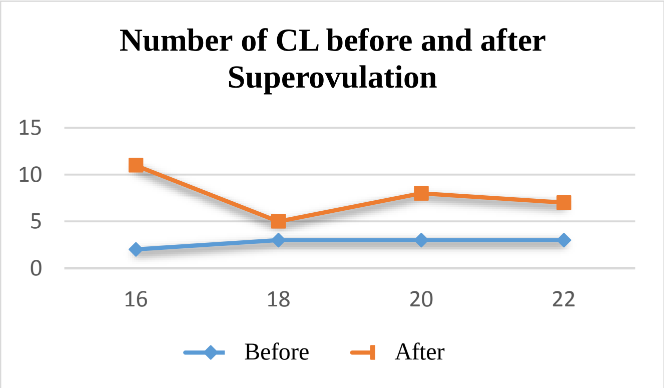

Figure 1. Number of CL before and after superovulation.

Figure 1 shows that after injection of PGF2α, the number of both CL increased. This is due to the superovulation treatment. Based on the data show that the highest distribution of CL is in the right ovary. This result follows Borghese (2010), who stated that there is more CL distribution in the right ovary compared to the left ovary. Right ovary activity is more active than the left ovary. Accordingly, Geres et al. (2011) stated that the right ovary is larger and more effective than the left ovary because of differences in ovarian activity, physiological differences in buffalo reproductive organs, and anatomical differences affecting ovarian venous blood flow to the ovaries. Differences in the number of CL produced by donor buffalo indicate that each buffalo had a different response to hormone treatment (Hafez, 2008).

Onset and duration of estrous

The statistical analysis of the onset of estrous in donor buffaloes shows no significant difference (P>0.05). The average onset and duration of estrous in the swamp buffaloes were 27.75 and 32.50 h. In swamp buffaloes without long estrous synchronization, the average onset and estrous duration are 20-28 h and 325-36 h (Ismaya, 2014). According to Yendraliza et al. (2012), swamp buffaloes show differences in estrous length because they are not all willing to copulate simultaneously. The estrous duration is influenced by several factors, including nature, season, age, temperature, feeding pattern, and livestock's response (Feradis, 2010). Duration of estrus is the time shown by swamp buffalo, with the first range showing signs of estrous and loss of estrus signs in swamp buffalo. After the superovulation treatment and injection of PGF2α were completed, all donor buffaloes showed signs of total estrous because all donor buffaloes were in their luteal phase, which the CL functions (Table 2).

Tabel 2. Average Estrous Onset (Hours) and duration (Hours) in The Swamp Buffalo with Different Doses

Dosages of GnRH (ml/buffalo)	Estrous onset (h)	Estrous duration (h)
A. 320 mg/ 16 ml	33.0	38.5
B. 360 mg/ 18 ml	21.0	40,5
C. 400 mg/ 20 ml	25.5	25.0
D. 440 mg/ 22 ml	31.5	26.0
Average	27.75	32.50

Note: Data presented as the mean of 4 replicates with Different Doses show no significance (P >0.05)

The estrous symptoms were marked by the changes in the outside reproductive organs (vulva) that turned red and swollen, discharge of mucus that had colored nodes; also mucus was stuck to the tail and rump, the change in temperature and pH of the vaginal (Barile et al. 2015) whereas behavioral estrous was marked by the habit of the buffaloes which often wagged their tails, they were also agitated and aggressive to other buffalo (Jessie et al. 2016). However, some buffaloes did not show estrous as they exhibited silent heat. This may be dues to internal factors of the buffaloes such as feeding pattern and environmental factors (Busch et al. 2008)

4. CONCLUSION

It is concluded that the administration of FSH with various levels of doses in swamp buffalo shows a superovulation response with an average of 56.25%. Administration of doses of 320 mg/16 ml, 360 mg/18 ml, 400 mg/20 ml, and 440 mg/22 ml FSH to swamp buffaloes showed the swamp's buffaloes, but a dose of FSH 320 mg gave the best superovulation response. Superovulation treatment with different doses of FSH yielded results that were not significantly different. The average number of corpus luteum, onset, and estrous in the swamp buffaloes were 2.75, 27.75, and 32.50 h.

REFERENCES

[1] Afriani, T. (2015). Penerapan Teknologi Reproduksi Pada Sapi. Padang: Andalas University Press.

[2] African, T., James, H., Purwanti, E., Ferdinand, R., Arif, R., Jaswandi, & Mangku, M. (2018). Reproductive Technology in Buffalo. Padang: Andalas University Press.

[3] Arum, W. P., Siregar, T. N., & Melia, J. (2013). Efek Pemberian Ekstrak Hipofisa Sapi Terhadap Respons Superovulasi Sapi Aceh. Jurnal Medika Veterinaria, 7(2).
[4] Barile, V. L. (2005). Improving Reproductive Efficiency in Female Buffaloes. Livestock Production Science, 92(3), 183-194.
[5] Barile, V. L., Terzano, G. M., Pacelli, C., Todini, L., Malfatti, A., & Barbato, O. (2015). LH Peak and Ovulation after Two Different Estrus Synchronization Treatments in Buffalo Cows in the Daylight-Lengthening Period. Theriogenology, 84(2), 286-293.
[6] Baruselli, P. S., de Carvalho, J. G. S., Elliff, F. M., da Silva, J. C. B., Chello, D., & de Carvalho, N. A. T. (2020). Embryo Transfer in Buffalo (Bubalus Bubalis). Theriogenology.
[7] Bearden, H. J., & Fuquay, J. W. (1984). Applied Animal Reproduction. Reston Publishing Company, Inc.
[8] Borghese, A. (2010, April). Development and Perspective of Buffalo and Buffalo Market in Europe and Near East. In Proc. 9th World Buffalo Congress, Buenos Aires (pp. 25-28).
[9] Busch, D. C., Schafer, D. J., Wilson, D. J., Mallory, D. A., Leitman, N. R., Haden, J. K., ... & Patterson, D. J. (2008). Timing of Artificial Insemination in Postpartum Beef Cows Following Administration of the CO-Synch+ Controlled Internal Drug-release Protocol. Journal of animal science, 86(7), 1519-1525.
[10] Carvalho, N. A. T., Soares, J. G., Porto Filho, R. M., Gimenes, L. U., Souza, D. C., Nichi, M., ... & Baruselli, P. S. (2013). Equine Chorionic Gonadotropin Improves The Efficacy of A Timed Artificial Insemination Protocol in Buffalo During the Nonbreeding Season. Theriogenology, 79(3), 423-428.
[11] Chaikhun, T., Hengtrakunsin, R., De Rensis, F., Techakumphu, M., & Suadsong, S. (2012). Reproductive and Dairy Performances of Thai Swamp Buffaloes Under Intensive Farm Management. The Thai Journal of Veterinary Medicine, 42(1), 81-85.
[12] De Rensis, F., & Lopez-Gatius, F. (2007). Protocols for synchronizing estrus and ovulation in buffalo (Bubalus bubalis): A review. Theriogenology, 67(2), 209-216.
[13] Feradis, M. P. (2010). Bioteknologi Reproduksi Pada Ternak. Bandung: Alfabeta.
[14] Gereš, D., Ževrnja, B., Žubčić, D., Zobel, R., Vulić, B., Staklarević, N., & Gracin, K. (2011). Asymmetrical Functional Activities of Ovaries and Tubular Part of Reproductive Organs of Dairy Cows. Veterinarski arhiv, 81(2), 187-98.
[15] Gimenes, L. U., Ferraz, M. L., Fantinato-Neto, P., Chiaratti, M. R., Mesquita, L. G., Sá Filho, M. F., ... & Baruselli, P. S. (2015). The Interval Between The Emergence of Pharmacologically Synchronized Ovarian Follicular Waves and Ovum Pickup Does Not Significantly Affect in Vitro Embryo Production in Bos Indicus, Bos Taurus, and Bubalus Bubalis. Theriogenology, 83(3), 385-393.
[16] Hafez, E. S. E. (2008). Preservation and Cryopreservation of Gamet and Embryos in Reproduction Farm Animal. ESE Hafez and B. Hafez. Maryland: Lippincott Williams & Wilkins
[17] Hasler, J. F. (1992). Current Status and Potential of Embryo Transfer and Reproductive Technology in Dairy Cattle. Journal of dairy science, 75(10), 2857-2879.
[18] Hermawan, E. (2019). Community Empowerment through Management of Village Funds Allocation in Indonesia. International Journal of Science and Society, 1(3), 67 - 79. https://doi.org/10.200609/ijsoc.v1i3.30
[19] Husaini, A. (2019). Arab Spring: Islam in the Political Revolution and Middle Eastern Development. International Journal of Science and Society, 1(4), 44-53. https://doi.org/10.200609/ijsoc.v1i4.152

[20] Ismaya, I. (2014). Bioteknologi Inseminasi Buatan Pada Sapi dan Kerbau (Biotechnology of Artificial Insemination on Cattle and Buffalo). Yogyakarta: Gadjah Mada University Press.
[21] Jodiansyah, S., Imron, M., & Sumantri, C. (2013). Tingkat Respon Superovulasi dan Produksi Embrio in Vivo dengan Sinkronisasi CIDR (Controlled Internal Drug Releasing) Pada Sapi Donor Simmental. Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan, 1(3), 184-190.
[22] Madan, M. L., Das, S. K., & Palta, P. (1996). Application of Reproductive Technology to Buffaloes. Animal Reproduction Science, 42(1-4), 299-306.
[23] Maddison, J. W., Rickard, J. P., Mooney, E., Bernecic, N. C., Soleilhavoup, C., Tsikis, G., ... & de Graaf, S. P. (2016). Oestrus Synchronisation and Superovulation Alter The Production and Biochemical Constituents of Ovine Cervicovaginal Mucus. Animal reproduction science, 172, 114-122.
[24] Nilchuen, P., Rattanatabtimtong, S., & Chomchai, S. (2011). Superovulation with Different Doses of Follicle Stimulating Hormone in Kamphaeng Saen Beef Cattle. Songklanakarin J Sci Technol, 33(6), 679-683.
[25] Pedersen, L. D., Kargo, M., Berg, P., Voergaard, J., Buch, L. H., & Sørensen, A. C. (2012). Genomic Selection Strategies in Dairy Cattle Breeding Programmes: Sexed Semen Cannot Replace Multiple Ovulation and Embryo Transfer as Superior Reproductive Technology. Journal of Animal Breeding and Genetics, 129(2), 152-163.
[26] Perera, B. M. A. O. (2011). Reproductive Cycles of Buffalo. Animal Reproduction Science, 124(3-4), 194-199.
[27] Rabidas, S. K., & Gofur, M. R. (2017). Synchronization of Estrus Using Ovsynch Protocol and Fixed Timed Artificial Insemination (FTAI) in Indigenous Dairy Buffaloes: An Effective Buffalo Breeding Program in Bangladesh. Asian Journal of Biology, 1-8.
[28] Rahman, M. R., Rahman, M. M., Khadijah, W. W., & Abdullah, R. B. (2014). Comparison of Superovulatory Effect of Equine Chorionic Gonadotrophin and Follicle Stimulating Hormone on Embryo Production in Crossbred (Boer A-Katjang) Goats. Pakistan Journal of Zoology, 46(3).
[29] Redhead, A. K., Siew, N., Lambie, N., Carnarvon, D., Ramgattie, R., & Knights, M. (2018). The Relationship Between Circulating Concentration of AMH And LH Content In The Follicle Stimulating Hormone (FSH) Preparations on Follicular Growth and Ovulatory Response To Superovulation in Water Buffaloes. Animal reproduction science, 188, 66-73.
[30] Safari, A., Hossein-Zadeh, N. G., Shadparvar, A. A., & Arpanahi, R. A. (2018). A Review on Breeding and Genetic Strategies in Iranian Buffaloes (Bubalus Bubalis). Tropical Animal Health and Production, 50(4), 707-714.
[31] Sakaguchi, K., Mayhem, E. R. S., Tilwani, R. C., Yanagawa, Y., Katagiri, S., Atabay, E. C., ... & Nagano, M. (2019). Effects Of Follicle-Stimulating Hormone Followed by Gonadotropin-Releasing Hormone on Embryo Production by Ovum Pick-Up and in Vitro Fertilization in The River Buffalo (Bubalus bubalis). Animal Science Journal, 90(5), 690-695.
[32] Setiadi, P. L., Supriatna, I., & Boediono, A. (2005). Follicle Development After Gonadotrophin Treatment in Garut Sheep for Laparoscopic Ovum Pick Up. J. Agri Rur Dev in the tropic and subtropics, 83, 153-158.
[33] Sianturi, R. G., Purwantara, B., Supriatna, I., & Amrozi, P. S. (2012). Optimasi Inseminasi Buatan pada Kerbau Lumpur (Bubalus Bubalis) Melalui Teknik Sinkronisasi Estrus dan Ovulasi. JITV, 17(2), 92-99.

[34] Silva, J. C. C., Alvarez, R. H., Zanenga, C. A., & Pereira, G. T. (2018). Factors Affecting Embryo Production in Superovulated Nelore Cattle1. Animal Reproduction (AR), 6(3), 440-445.
[35] Singh, N., Dhaliwal, G. S., Malik, V. S., Dadarwal, D., Honparkhe, M., Singhal, S., & Brar, P. S. (2015). Comparison of Follicular Dynamics, Superovulatory Response, and Embryo Recovery between Estradiol Based and Conventional Super-stimulation Protocol In Buffaloes (Bubalus Bubalis). Veterinary World, 8(8), 983.
[36] Statistik, B. P. (2017). Populasi Ternak Provinsi Sumatera Barat Menurut Kabupaten Kota Tahun 2013-2017.
[37] Suardi. (2012). Fisiologi Reproduksi Hewan. Padang: Andalas University Press.
[38] Velazquez, M. A. (2011). The role of nutritional supplementation on the outcome of superovulation in cattle. Animal reproduction science, 126(1-2), 1-10.
[39] Yendraliza, B. P., Zespin, Zaituni, U., & Jaswandi. (2012). Post-Partum Reproductive Appearance of Buffalo at Various Levels of GnRH and Synchronized with PGF2α. JITV, 17(2), 107-111.