Research Article

Semen Quality of Ongole Crossbred Bull with Gonadotropin Realizing Hormone (GnRH) Treatment

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Abstract | Administration GnRH to bulls is expected to improve semen quality. This study aims to determine the effect of giving the hormone GnRH in various doses on the quality of semen in Ongole Crossbreed (OC) bulls. The study used 18 OC bulls with three GnRH treatments, A (one dose/5ml/animal), B (half-dose/2.5ml/animal), and C without GnRH. The GnRH was administered once by intramuscular (IM) injection. Semen collection was carried out for 16 weeks; divided into two periods, namely 8 and 16 weeks after GnRH administration. Parameters that were measured include volume, acidity, color, consistency, mass motility, individual motility, spermatozoa concentration, viability, and libido. Data were analyzed using the ANOVA test. The results showed that the volume and pH of semen did not differ in all treatments, but treatment A showed cream color and thick consistency over treatments B and C. The mass motility and sperm concentration of treatment C with the dose and period of GnRH administration resulted in the lowest values significantly different (P<0.05) than treatments A and B, so it affects the total spermatozoa and the total motile spermatozoa. Sperm viability in one dose of treatment A resulted in the highest value significantly different (P<0.05) than treatment B and C with spermatozoa viability of 78.42±2.46% (A), 76.14±1.52% (B), and 65.57±8.26% (C) in the 16 weeks. Nevertheless, the abnormalities and libido between treatments and different periods did not show any difference. It was concluded that the administration of GnRH at a dose of 5 ml/IM can increase the mass motility, individual motility, sperm concentration, total spermatozoa, total motility of spermatozoa, and viability in Ongole Crossbred bulls.

Keywords | The Ongole Crossbred bulls, GnRH, Semen quality

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INTRODUCTION

The success of pregnant cows depends on the level of bull's and cow's fertility, and mating management. Problems with bulls being used as bulls include low semen quality and libido. Low semen quality has an impact on decreasing the level of conception of cows which causes a decrease in the population and income of farmers. The limited number of superior bulls, both as natural bulls and as a source of semen, leads to long calving intervals which ultimately results in low cattle productivity. Factors that affect semen quality and libido include age (Azzahra et al., 2016; Prastowo et al., 2018), season (Aisah et al., 2017), ejaculation frequency (Sholikah et al., 2018), live weight (Affandhy et al., 2018; Nugraha et al., 2019), exercise and nutrition (Ratnawati et al., 2015). Baharun et al. (2017) stated that the libido and quality of Bali bulls according to the Indonesian National Standard was only around

26.4%, while the semen quality of OC bulls in Kebumen in the field experienced fluctuations in semen quality with spermatozoa concentrations between 700-1700 million/ml (Affandhy et al., 2013). Affandhy et al. (2018) reported that OC bulls with live weight >300 kg showed average individual mass motility of ++ and a spermatozoa concentration of 370 million/ml.

An effort to increase bull fertility can be through increasing semen quality and libido, including through regular exercise giving a combination of herbs and vitamins (Affandhy et al., 2017; Ratnawati et al., 2012) or by injection of HCG and GnRH (El-Khawaga et al., 2012;; Devkota et al., 2011; Samir et al., 2015). Gonadotropin Realizing Hormone plays a role in stimulating the anterior pituitary to secrete LH and FSH which will stimulate the testicular Ley dig cells to secrete testosterone and estrogen and Sertoli cells to secrete inhibit and activin. Testosterone and estrogen produced by Ley dig cells give negative feedback both to the hypothalamus and anterior pituitary which will suppress the secretion of GnRH from the hypothalamus and FSH, and LH from the anterior pituitary. Similarly, inhibit and activin produced by Sertoli cells are specific negative feedback on the anterior pituitary, testosterone produced by Ley dig cells will bind to Androgen receptors (AR) in Sertoli cells which will secrete androgen binding protein (ABP), and inhibit and help in the process of formation of spermatozoa (Hasbi and Gustina, 2018).

Research on giving GnRH hormones with various doses or combinations to bulls in Indonesia is still rarely done, so far it has been mostly done on cows to support estrus or ovulation synchronization programs (Affandhy et al., 2013). This study aims to determine the effect of giving the hormone GnRH in various doses on the quality of semen in OC bulls.

MATERIALS AND METHODS

This research has been conducted and approved by the Experimental Animal Welfare Commission (EAWC) for the supervision of the use of research livestock with the number Balitbangtan/Lolitsapi/ Rm/ 06/ 2020.

Time and Location of Study

Research data collection was carried out on Ongole Crossbreed (OC) bulls in the experimental individual of the Beef Cattle Research Institute (BCRI) in Grati District, Pasuruan Regency, East Java, Indonesia, starting from July 2020 to January 2021. Fresh semen was collected in the cattle crush and a semen quality analysis was carried out. in the BCRI reproductive laboratory.

Research Materials

This study used 18 Ongole Crossbred bulls at the age of 3-5 years with a motility condition of ≤40% (Contri et al., 2012). Semen was collected using an artificial vagina. Analysis of the quality and volume of semen using a set of reproductive test equipment (artificial vagina, hemocytometer, pH paper, microscope, water bath, measuring cup, object-glass, cover glass, counter, and so on). The GnRH ingredient contains Buserelin acetate 0.0084 mg, excipients q.s.ad 1 ml (Conceptase ®). There were three GnRH treatments in this study, namely A (one dose), B (half-dose), and C without GnRH. GnRH is administered once by intramuscular (IM) injection. Semen collection was carried out for 16 weeks; divided into two periods, namely 8 and 16 weeks after GnRH administration. One dose (5.0ml/animal) and half-dose (2.5ml/animal) of GnRH were administered. The experimental design used a completely randomized design (CRD) with three doses of GnRH treatment.

Collection and Evaluation of Semen

Semen collection of bulls was carried out once a week for 16 weeks which was divided into two observation periods, namely 8 and 16 weeks after being given GnRH injection and GnRH administration based on the spermatogenesis cycle (Contri et al., 2012). Each treatment used six bulls. The feeding of bulls was based on the standard of feeding for bulls at BCRI, which consisted of a crude protein of 11-12% of 3% DM based on the bull's live weight.

Semen collection was carried out in the morning between 07.00-08.00 with an interval of once a week for 16 weeks using an artificial vaginal device (IMV®). Measurement of the volume of semen was carried out using a scaled tube as well as an assessment of the semen color, consistency, and pH of the semen. Evaluation of semen quality using CASA which includes mass motility, and individual motility. Calculation of the concentration of spermatozoa used a spectrophotometer. Spermatozoa concentration using a spectrophotometer was calculated based on 3960 µl of Na Cl (0.9%) solution added with 40 µl of semen (IMV®). Smear preparations for viability analysis by using eosin negrosin; then examined using CASA with 1000 times magnification.

Semen color observation is based on a score of 1 (watery); a score of 2 (milky); and a score of 3 (creamy) (Maskur et al., 2020), and the consistency assessment was based on a score of 1 (thin), a score of 2 (medium) and a score of 3 (thick) (Suhardi et al., 2020). For mass motility research based on a score of 0 (there is no movement), 1+ (there is a group of sperm that move slightly), 2++ (there is a group of sperm that moves a lot, 3+++ (there is a group of sperm that moves very quickly, and 4++) ++ (fast rotating movement like cloudy/cloudy) (Cenariu et al., 2018).

Assessment of the percentage of motility using CASA with a magnification of 10 x 10 based on progressive motion (Maskur et al., 2020). The counting of total spermatozoa was calculated based on volume x concentration of spermatozoa and total motile spermatozoa were calculated based on volume x concentration of spermatozoa x percentage of motile individuals using CASA in IBCRS (Affandhy et al., 2018).

Evaluation of Libido

The libido evaluation was carried out at each semen storage and the end of the study. The libido of bulls was measured base on the time of bull from approaching the teaser cow to mounting in units of time.

Statistical Analysis

Parameters measured include the semen quality (mass movement, motility, spermatozoa concentration, viability), color, pH, consistency, the volume of semen, and libido of bulls. The data were analyzed by using ANOVA.

RESULTS AND DISCUSSION

Evaluation of semen quality in OC bulls with macroscopic different doses of GnRH consisting of volume, color, pH, and consistency semen is presented in Table 1.

Macroscopic Semen Quality

The results showed that there was no significant difference (P>0.05) between all treatments and periods of 8 and 16 weeks after injection of GnRH. This is following the research results of Sajjad et al. (2007) who reported that GnRH injection in male buffalo did not affect semen volume and pH. Waheed et al. (2018) stated that administration of GnRH (Theriogon®) did not effect to pH of semen of bull, meanwhile, the volume of semen was higher, Injection of gonadotropin-releasing hormone (GnRH) can increase the multiplication of Sertoli and Ley dig cell, than increasing testicular weight, sperm production, sexual maturity and germ cell count (Kumar et al., 2015; Doroteu et al., 2021). The semen in treatment A showed the best color and consistency of semen compared to treatments B and C (Table 1). Gonadotropin Realizing Hormone with one dose in the period 8 and 16 weeks after administration of GnRH produces the best color and consistency of semen. The color of bull bovine semen can be influenced by the consistency and concentration of spermatozoa (Yendraliza et al., 2019). Setiono et al. (2015) stated that the color criteria for good bull semen to be processed into frozen semen was milky white. Administration of GnRH to Ongole Crossbred bulls using one dose of GnRH (5 ml/IM) was able to maintain semen with a creamy color and thick consistency. Giving GnRH resulted in a better color of semen in Simmental or Limousine bulls than without GnRH treatment which produced a milky white color and medium consistency (Denilisvanti et al., 2017).

Microscopic Semen Quality

Evaluation of semen quality in OC bulls with microscopically different doses of GnRH including mass motility, individual motility, the concentration of spermatozoa, viability, and abnormalities are presented in Table 2 and 3.

Mass and individual motility, and sperm concentration in Ongole Crossbreed bulls in treatment C (without GNRH administration) at different doses and periods of GnRH administration showed the lowest values significantly (P<0.05) compared to the values of treatments A and B as well as with the values of total spermatozoa and total motile spermatozoa at the 16 weeks of GnRH administration were significantly different (P<0.05) with the 8-week administration period, namely in treatment A, which was one dose of GnRH administration (Table 2). Administration of one dose of GnRH at 8 weeks resulted in the best spermatozoa concentrations (3750.53±309.65 million/ml), total spermatozoa (668.50±235.77 million/ml), and total motile spermatozoa (2668.50±235.77 million). (Table 2). These results indicate that the treatment with the addition of GnRH can maintain mass motility and individual motility and is more efficient for Treatment B. Mass motility and individual motility are still closely related and correlated with spermatozoa concentration (Talluri et al., 2016). Based on the calculation of the total motility of spermatozoa, the highest total motility was 2668.50 ±235.77 million in treatment A with a period of 8 weeks of GnRH administration, which showed a significant difference (P<0.05) compared to treatments B, and C, respectively, the total value of motile spermatozoa was 1,769.09±250.96 and 1,161.40±181.36 million (Table 2). This is in line with the report of Fitrayadi et al. (2021) which stated that frozen semen production was influenced by semen volume and spermatozoa concentration, and the administration of herbal stimulants. Administration of oral GnRH stimulants at 100 mg per kg/lightweight also increased libido and total spermatozoa motile (Waheeb et al., 2018). Subcutaneous administration of GnRH can increase spermatozoa concentration and spermatozoa characteristics after eight weeks after hormone therapy (Contri et al., 2012). Administration of exogenous GnRH was shown to improve sexual behavior and increase the quality of frozen/thawed spermatozoa in fertile stallions during the non-breeding season (Sieme et al., 2004). Thus, hypothalamus gonadotropin-releasing hormone (GnRHR) can control the activity of the hypothalamus-pituitary-gonadal axis and play a key role in animal reproductive performance so that it can have an impact on improving semen

Table 1: Average volume, color, pH, and consistency semen in Ongole Crossbred bulls

Treatments		GnRH Adminstration Period	
		8 weeks	**16 weeks**
Volume (ml)	A	2.18±0.29	3.27±0.61
	B	3.24±0.74	2.74±0.46
	C	2.24±0.91	3.55±0.84
pH	A	6.55±0.10	6.66±0.07
	B	6.83±0.10	6.64±0.07
	C	6.93±0.07	6.96±0.18
Color	A	creamy	creamy
	B	milky	milky
	C	watery	watery
Consistency	A	thick	thick
	B	medium	medium
	C	thin	thin

Note: A: one dose of GnRH injection; B: half-dose of GnRH injection and C without GnRH injection as control

Table 2: Average mass motility, individual motility, the concentration of spermatozoa, total spermatozoa, and total motile spermatozoa in Ongole crossbred bulls

Treatments		GnRH Adminstration Period	
		8 weeks	**16 weeks**
Mass Motility	A	2.05±0.10^{bc}	2.29±0.08^{b}
	B	1.64±0.20 ^{b}	2.33±0.20^{b}
	C	0.86±0.25^{a}	1.03±0.07^{a}
Individual Motility (%)	A	70.53±3.85^{c}	70.53±3.85^{c}
	B	59.96±5.83^{b}	59.96±5.83^{b}
	C	49.89±2.49^{a}	49.89±2.49^{a}
Sperm Concentration (million/ml)	A	961.33±102.03^{bc}	1,146.95±156.42^{c}
	B	675.02±64.83^{ab}	977.67±154.54^{bc}
	C	424.02±61.85^{a}	574.66±74.90^{a}
Total Spermatozoa (million/ml)	A	1,146.95±156.42^{c}	3,750.53±309.65^{c}
	B	977.67±154.54^{bc}	2,678.82±324.82b^{b}
	C	574.66±74.90^{a}	2,040.04±619.62b^{a}
Total Motile Spermatozoa (millions)	A	1,478.10±243.76^{b}	2,668.50±235.77^{c}
	B	1,311.36±312.31^{b}	1,769.09±250.96^{b}
	C	473.86±103.97^{a}	1,161.40±181.36^{a}

Note: A: one dose of GnRH injection; B: half-dose of GnRH injection and C without GnRH injection as control; ^{abc}different superscripts on the same line and colum showed significant differences (P<0.05).

Table 3: Average viability, spermatozoa abnormalities, and libido in Ongole crossbred bulls

Treatments		GnRH Adminstration Period	
		8 weeks	**16 weeks**
Sperm viability (%)	A	78.24±3.86^{c}	78.42±2.46^{c}
	B	69.85±5.61^{ab}	76.14±1.52^{ab}
	C	60.64±5.57^{a}	65.57±8.26^{ab}
Sperm abnormalities (%)	A	12.71±3.30	10.80±2.27
	B	11.99±3.23	18.86±3.17

	C	25.67±10.50	11.98±3.49
Libido (minute)	A	10.80±2.27	3.24±0.61
	B	18.86±3.17	2.44±0.24
	C	11.98±3.49	3.02±0.48

Note: A: one dose of GnRH injection; B: half-dose of GnRH injection and C without GnRH injection as control; [abc]different superscripts on the same line and colum showed significant differences (P<0.05).

quality (Wang et al., 2020), and this GnRH gene can also be used as a genetic marker associated with semen quality in buffalo because good semen has specific characteristics (Mahmoud et al., 2020).

Viability, spermatozoa abnormalities, and libido

Sperm viability in treatment A at one dose showed a significantly higher value (P<0.05) than in treatments B and C with the value of sperm viability in each treatment A (78.42±2.46%), B (76.14±1.52%), and C (65.57±8.26%) over 16 weeks. There was no difference in abnormality of spermatozoa and libido in all treatments and periods of GnRH administration (Table 3) with the libido score at the end of the study (16 weeks after giving GnRH for 24 hours in all treatments (Figure 1). The administration of GnRH at one dose increased the viability of spermatozoa at better than half-doses and without GnRH. This is almost the same as the treatment with the combination of GnRH and Zinc methionine resulting in significantly different spermatozoa viability between all treatments and periods. Spermatozoa abnormalities with the administration of one dose of GnRH ranged from 9,2-12.9% (less than 20%), thus fulfilling the semen quality requirements of bulls. Purwantara et al. (2010) stated that the level of morphological abnormalities of spermatozoa of Friesian Holstein (FH) bulls in samples collected from three Artificial Insemination Centers (AI) in Indonesia is in the normal range, which is between 1.00 to 8.40%. Thus, OC bulls that have spermatozoa viability of ≥70% are still normal and can be used as compensation in determining the normal viability of each ejaculate bull (Perry, 2021).

Giving GnRH to PO bulls had no effect on libido between doses and periods of GnRH administration (Table 3). This is following the results of the study Malmren et al. (2001) stated that the effective stimulating of GnRH was in an individual variation in the responses among the stallions and, further, libido was not suppressed. This is in line with the results of the study by Monaco et al. (2015) stated that giving GnRH can increase testosterone concentration, libido, and spermatozoa concentration (Waheeb et al., 2018).

Libido is a characteristic that must be considered and is highly correlated with herd reproduction parameters (Tores-Aburto et al., 2020). Gonadotropin Realizing

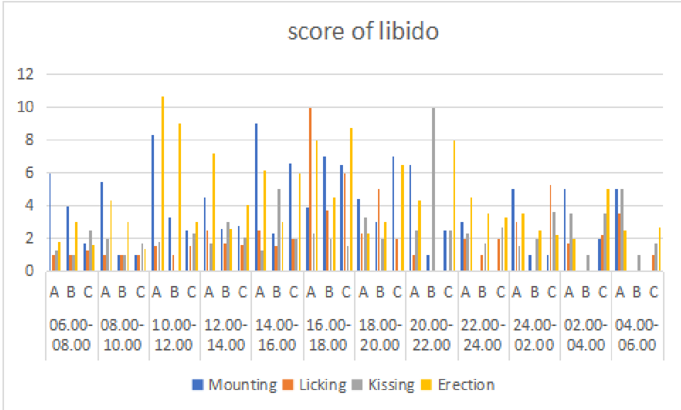

Figure 1: The value of libido

Hormone treatment to increase the scrotal surface temperature of males avoids environmental temperature stress (Gabor et al., 2001) which causes abnormal spermatozoa levels (Rahman et al., 2018). Oral GnRH administration of 100 mg per kg/lightweight resulted in increased libido and total spermatozoa motile (Waheeb et al., 2018). Giving GnRH stimulants can be used to increase sexual behavior or libido as well as molecular mechanisms in male reproduction. The previous study by El-Khawaga et al. (2011) stated that injection of GnRH at dose 12 could maximize semen quality and libido of pubertal buffalo bulls. Testosterone secretory capacity before and after GnRH treatment can change plasma concentrations of LH and testosterone in bulls aged 20 months with normal semen (Sakase et al., 2018). Nevertheless, the study by Mondal et al. (2019) stated that LH hormones and peripheral rhythm of testosterone could not be used to detect libido problems in Zebu bulls.

CONCLUSION

Administration of GnRH at a dose of 5 ml/IM can increase the mass motility, individual motility, sperm concentration, total spermatozoa, total motility of spermatozoa, and viability in Ongole Crossbred bulls.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

LA planned, designed the research, analyzed all data, and drafted the manuscript, ML, DR, YW, and MP provided and help in the research. All authors discussed the results and contributed to the final manuscript.

REFERENCES

Affandhy L. Y. Adinata, Aryogi (2013). Evaluation of the semen quality of PO Kebumen bulls in Kebumen Regency, Central Java Province. J. Sain Dasar. 2 (2): 49-55. https://doi.org/10.21831/jsd.v2i2.3299

Affandhy L., D. Ratnawati, M. Luthfi (2017). The effect of giving a combination of traditional herbal medicine on the semen quality and libido of Ongole Crossbreed. Trad. Med. J. 22 (2): 84-94. https://doi.org/10.22146/tradmedj.27918

Affandhy L., H.P. Fitrayadi, M. Luthfi, Y. Widyaningrum (2018). Effect of live weight on libido, sperm quality, testosterone, and luteinizing hormone in replacement stock of Ongole Grade bull. J. Indonesian Trop. Anim. Agric. 43(4):352-360. https://doi.org/10.14710/jitaa.43.4.352-360

Aisah S., N. Isnaini, dan S. Wahyuningsih (2017). Kualitas semen segar dan recovery rate sapi Bali pada musim yang berbeda. J. Ilmu-Ilmu Peternakan. 27 (1): 63 – 79. https://doi.org/10.21776/ub.jiip.2017.027.01.06.65

Azzahra F.Y., E.T. Setiatin, D. Samsudewa (2016). Evaluation of Motility and Live Percentage of Fresh Semen of PO Kebumen Young Bulls. J. Sain Peternakan Indonesia. 11 (2): 99-107. https://doi.org/10.31186/jspi.id.11.2.99-107

Baharun A., H. Belli, TM Hine (2017). Characteristics of young male Bali cattle on smallholder farms in Merbaun village, Kupang district. J. Peternakan Nusantara. 3(1): 11-16. https://doi.org/10.30997/jpnu.v3i1.850

Cenariu M., E. Pall, M. Borzan, L. Bogdan, I Groza. (2018). Advanced Techniques of Bovine Semen Analysis. Bulletin UASVM Vet. Med. 75(1): 59-65. https://doi.org/10.15835/buasvmcn-vm:004317

Contri A., A. Gloria, L. Wegherb, A. Carluccioa (2012). Successful use of a gonadotropin-releasing hormone (GnRH) analog for the treatment of tertiary hypogonadism (GnRH deficiency) in a 5-year-old Belgian Blue bull. Vet. Quart., 32 (1): 51–54. https://doi.org/10.1080/01652176.2012.677866

Denilisvanti B. Muada, Umar Paputungan, Manopo J. Hendrik*, Santie H. Turangan (2017). Characteristics of fresh semen in the Limousine and Simmental at Lembang Insemination Center. J. Zootek. 37 (2): 360-369.

Dethan A.A., kustono dan H. Hartadi (2010). Kualitas dan kuantitas sperma kambing Bligon jantan yang diberi pakan rumput gajah dengan suplementasi tepung darah. Buletin Peternakan. 34 (3): 145-153.

Devkota B., Ken-Inch Takahashi S., Matsuzaki M., Matzui A., Miyamoto N., Yamagishi T., Osawa T., Hashizume Y., Izaike, Yoh-Inchi Mitake (2011). Basal Levels and GnRH-induced Responses of Peripheral Testosterone and Estrogen in Holstein Bulls with Poor Semen Quality. J. Reprod. Dev. 57: 373–378. https://doi.org/10.1262/jrd.10-136T

Doroteu E.M., J.H.M.Viana, J.A.F. Junior, J.T.A. Macedo, R.A. Oliveira, P.M.O. Pedroso (2021). Effect of a single or two doses of an anti-GnRH vaccine on testicle. Trop. Anim. Health Prod. 53: 153. https://doi.org/10.1007/s11250-021-02600-x

El-Khawaga A.R.M., Mohamed M.M. Kandiel, Gamal A. Sosa, Mahmoud E.A. Abou El- Roos, Alaa Ee. Abdel-Ghaffar, Abd El- Salam I. El Azab (2011). Effect of GnRH analog on libido and semen characteristics of puberal buffalo bulls. Benha Vet. Med. J. special issue (i): 28—34. https://doi.org/10.3923/ajava.2018.218.225

Fitrayadi H.P., M. Luthfi dan L. Affandhy (2021). Analisis ekonomi lama penggemukan sapi jantan Peranakan Ongole (PO) berdasarkan bobot badan ternak. J. Madura Ranch. 6(1):1-8.

Gabor G., J.P. Kastelic R. B. Cook R. G. Sasser L.F.C. Brito J.V. Csik G. H. Coulter I. Gyorkos (2001). Effects of GnRH treatment on scrotal surface temperatures in bulls. Canadian J. Vet. Res. 65 (1):60-63. https://europepmc.org/article/med/11227197

Hasbi, S. Gustina (2018). Androgen regulation in spermatogenesis to increase male fertility. Wartazoa. 28 (1):013-022. http://dx.doi.org/10.14334/wartazoa.v28i1.1643

Kumar B.S.B., S. Pandita, A. Sharma, V. Jadhav, S. Soren, B. Mili, A.H. Ganaie, N. A. Mir, N. Kumar, Vandna, A. Kumar (2015). Regulation of postnatal development of testes and its association with puberty and fertility - A review. Agric. Rev., 36 (4): 339-344. https://doi.org/10.18805/ag.v36i4.6671

Mahmoud K.Gh., A. M. Sakr, S. R. Ibrahim, A. S. Sosa, M. H. Hasanain, M. F. Nawito (2020). GnRHR gene polymorphism and its correlation with semen quality in Buffalo bulls (*Bubalus bubalis*). Iraqi J. Vet. Sci., 35 (2): 381-386. https://doi.org/10.33899/ijvs.2020.126886.1407

Malmren L, O Andresen, A M Dalin (2001). Effect of GnRH immunization on hormonal levels, sexual behavior, semen quality, and testicular morphology in mature stallions. Equine Vet. J. 33 (1):75-83. https://doi.org/10.2746/042516401776767340

Maskur, C. Arman, S. Said (2020).The Characteristics of Motile and Static Semen of Swamp Buffalo in Teaching and Experimental Farm Lombok. Indonesian J. Anim. Sci. Tech. 6 (2): 71-82.

Monaco D. M., Fatnassi B., Padalino L., Aub´e T., Khorchani M., Hammadi G. M (2015). Lacalandra. Effects of a GnRH administration on testosterone profile, libido, and semen parameters of dromedary camel bulls. Res. Vet. Sci., Elsevier, 102:212-216. https://doi.org/10.1016/j.rvsc.2015.08.011

Mondal S, Bhakat M, Singh A, Mohanty TK, Abdullah M (2019). Libido problem is untraceable through testosterone and luteinizing hormone rhythm in zebu breeding bulls. J. Anim. Health Prod. 7(3): 81-84. http://dx.doi.org/10.17582/journal.jahp/2019/7.3.81.84

Nugraha C.D., E. Herwijanti, I. Novianti, A. Furqon, W.A. Septian, W. Busono, S. Suyadi (2019) . Correlations between the age of Bali bull and semen production at National Artificial Insemination Center, Singosari – Indonesia. J. Indonesian. Trop. Anim. Agric., 44(3):258-265. https://doi.

org/10.14710/jitaa.44.3.258-265
Padalino B., D. Monaco, M. Fatnassi, L. Aubé, T. Khorchani, M. Hammadi, G. M. Lacalandra (2015). Effects of a GnRH administration on testosterone profile, libido, and semen parameters of dromedary camel bulls. Res. Vet. Sci., 102 (10): 212-216. https://doi.org/10.1016/j.rvsc.2015.08.011
Perry V.E.A (2021). The Role of Sperm Morphology Standards in the Laboratory Assessment of Bull Fertility in Australia. Front. Vet. Sci. 8:672058. https://doi.org/10.3389/fvets.2021.672058
Prastowo S., P. Dharmawan, T. Nugroho, A. Bachtiar, Lutojo, A. Pramono (2018). Fresh semen quality of Bali cattle (Bos javanicus) in different age groups. J. Ilmu Ternak. 18(1):1-7. https://doi.org/10.24198/JIT.V1811.17684
Purwantara B., R.I. Arifiantini, M. Riyadhi (2010). Sperm Morphological Assessments of Friesian Holstein Bull Semen Collected From Three Artificial Insemination Centers In Indonesia. J. Indonesian Trop. Anim. Agric. 35(2):90-94. https://doi.org/10.14710/jitaa.35.2.90-94
Rahman M.B., K. Schellander, N.L. Luceno, A.V. Soom (2018). Heat stress responses in spermatozoa: Mechanisms and consequences for cattle fertility. Theriogenology., 113: 102-112. https://doi.org/10.1016/j.theriogenology.2018.02.012
Ratnawati D., Luthfi M., Affandhy L (2012). Effect of traditional herbal supplementation on performance of PO bull. Proceeding Conference on Livestock Production and veterinary technology. Editors Elizabeth Wina et al., (2012) Indonesian Center For Animal Research and Development, Indonesian Agency For Agricultural Research and development, Ministry of Agriculture Republic of Indonesia: 91-96.
Ratnawati D, Y. Widyaningrum, A. Sulistya (2015). Perlakuan Exercise pada Sapi Jantan PO terhadap Peningkatan Kualitas Semen. Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner. Pusat Penelitian dan Pengembangan Peternakan. Badan Penelitian dan Pengembangan Pertanian, Bogor: 81-8.
Sajjad M., S. Ali, S. Akhter, N. Ullah (2007). Effect of Gonadotropin-Releasing Hormone on semen characteristics in Nili-Ravi buffalo bulls. Pakistan Vet. J., 27(3): 153-154.
Sakase, M,. W.W.P.N. Weerakoon, M.A. Hannan, N. Kohama, H. Tamada, N. Kawate (2018). LH and testosterone secretions in response to GnRH challenge in pubertal Japanese Black beef bulls with normal and abnormal semen. J. Vet. Med., 80 (12): 1829-1833. https://doi.org/10.1292/jvms.18-0151
Samir H., K. Sasaki, E. Ahmed, A. Karen, K. Nagaoka, M.El-Sayed, K. Taya dan G. Watanabe (2015). Pengaruh suntikan tunggal hormon pelepas gonadotropin (GnRH) dan human chorionic gonadotropin (hCG) pada aliran darah testis diukur dengan ultrasonografi doppler warna pada kambing Shiba jantan. J. Dokter Hewan Med. Sci. 77 (5):549-556. https://doi.org/10.1292/jvms.14-0633
Setiono N., Suharyati. S., Edy Santoso P (2015). Brahman bull's frozen semen quality with different doses of cryoprotectant glycerol in yolk tris sitrat diluent. J. Ilmiah Peternakan Terpadu. 3(2):61-69.
Sholikah N., A. Sutomo, N. P. Widiasmoro S. Wahjuningsih, A.P.A. Yekti, Kuswati, dan T. Susilawati (2018). Hubungan antara Tingkah Laku Seksual dengan Produksi Spermatozoa Sapi Brahman. Agripet. 18 (2):67-73. https://doi.org/10.17969/agripet.v18i2.12036
Sieme H., M.H.T.Troedsson, S.Weinrich, E.Klug (2004). Influence of exogenous GnRH on sexual behavior and frozen/thawed semen viability in stallions during the non-breeding season. Theriogenology. 61 (1):159-171. https://doi.org/10.1016/S0093-691X(03)00205-X
Suhardi R, N. Megawati, F. Ardhani, P. Summpunn, S. Wuthisuthimethavee (2020). Motility, Viability, and Abnormality of the Spermatozoa of Bali Bull with Andromed® and Egg Yolk-Tris Diluents Stored at 4 °C. Iranian J. Apply. Anim. Sci. 10(2), 249-256.
Talluri T.R, G. Mal, S.K. Ravi (2016). Biochemical components of seminal plasma and their correlation to the fresh seminal characteristics in Marwari stallions and Poiton jacks. Vet. World. 10 (2): 214-220. https://doi.org/10.14202/vetworld.2017.214-220
Torres-Aburto V. F., M. Barrientos-Morales, H. Hernández-Flores, A. Rodríguez-Andrade, P. Cervantes-Acosta, V. Landi, A. H. Beltran, B. Domínguez-Mancera (2020). Breeding soundness examination and herd proficiency of local genetic groups of bulls in tropical environment conditions in Veracruz, Mexic. Italian J. Anim. Sci. 19 (1): 840–855. https://doi.org/10.1080/1828051X.2020.1803773
Waheeb. R.S., M. Ashry, A.B.A. Ali, A. Gamal (2018). Amraw Effects of Oral Administration of Gonadotrophin Stimulant (Theriogon®) on Sexual Behavior and Semen Characteristics in Bulls. Asian J. Anim. Vet. Adv., 13 (3): 218-225. https://doi.org/10.3923/ajava.2018.218.225
Wang S., Y. Zhang, Y. Cheng, G Lu, R Yang, H. Geng, C. Wang, H. Li, T. Feng, S. Liu, L. Hao (2020). Association of SNPs in GnRH gene with sperm quality traits of Chinese water buffalo. Reprod. Domest. Anim., 55 (3): 384-392. https://doi.org/10.1111/rda.13634
Yendraliza H., Abadi R., Misrianti A., Ali dan, A. Effendi (2019). Identifikasi ukuran tubuh dan kualitas semen sapi kuantan jantan. JIPT. 7(1): 186 – 191. http://dx.doi.org/10.23960/jipt.v7i1.p186-191