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Stress Evaluation and Stress Response of Goat's Intracervical Insemination in Indonesia Smallholder Farms

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Abstract— *Goats artificial insemination (AI) in Indonesia is not as popular in smallholder farms as in cattle's AI because their success is low compared to natural mating (NM). The implementation of AI is carried out intracervical so that it triggers stress. The aim of the study was to measure the AI stress response and evaluate the success of intracervical goat AI in smallholder farms. Crossbred Ettawa goats were treated with NM and AI for the first time. Previously performed estrus synchronization. Measurement of physiological status and blood samples were taken before and after mating. Blood samples were used to measure the molecular stress response including Superoxide dismutase (SOD), Catalase (CAT), Glutathionperoksidase (GPx), Malondialdehyde (MDA), Neutrophils and Progesterone. Evaluation of mating success by calculating the conception rate, birth weight, litter size, weaning weight, progesterone. Data were analyzed statistically using a t-test. The results showed that the physiological status before and after mating was significantly different (P<0.05) except that the rectal temperature was not different (P>0.05). The activity of SOD, CAT, GPx, MDA, and neutrophil was different (P<0.05) before and after mating with a tendency to increase after mating. The conception rate of NM was 100%, AI was 20%, progesterone, birth weight, litter size, and weaning weight NM was different (P<0.05) compared to AI. The levels of progesterone, birth weight, litter size, and weaning weight in NM were higher than AI for the first time. Intracervical Goat's AI treatment gave a greater stress response with low success compared to natural mating*.

Keywords— *Artificial, Evaluation, Farm, Insemination, Smallholder*.

I. INTRODUCTION

Goat artificial insemination (AI) in Indonesia is not as popular in smallholder farms as AI in cattle. Goat's AI is lessattractive to farmers because their success is not better with natural mating (Prihatin et al., 2021). Whereas AI in smallholder farms is very much needed because theproductivity of local goats is relatively low compared to sub-tropical goats (Ciptadi et al. 2014). The low productivity of local goats is because they are not yet specialized in accordance with their production goals. The superiority of local goats lies in their adaptability and reproductive efficiency. According to Cseh et al. (2018), AI is an effective and efficient tool in implementing livestock breeding policies through improving the genetic quality of livestock. The success rate of AI in Indonesia is still low, especially in smallruminants. Sutama (2011) reported that the success of naturalmating in crossbred Ettawa goats was 84.2% while through AI was 40.9%. According to Inounu (2014) the success of intracervical goat AI 47.6%. Nuraini (2021) reported thesuccess of natural mating pregnancy in goats 73.4% and IA 21%.

The studies of efforts to increase the success of The Goat AI so far have been seen from only doe goats such as selecting productive doe with a good reproductive cycle, estrus synchronization, semen quality, placement of the rightsemen at the right time (Inounu, 2014). Publications of the success of AI in goats and sheep in field conditions in Indonesia have not yet been published, but Inounu (2014) predicts that the pregnancy rate is still less than 30%. On thelaboratory scale, the success of AI which is based on the conception rate is 45.45% while natural mating is 84.62% (Prihatin et al. 2021).

The implementation of goat AI which is often carried out on people's farms is intracervical AI. The implementation of intracervical goat IB is thought to stress livestock so that the success of pregnancy is low. Implementation of AI requires a minimum of two people. One person served as an inseminator and the other as a fixator for the head andperianal area of the goat. Goat fixation is done to make it easier to insert the insemination device. The fixator lifts the perianal area as well as the goat's hind legs up so that the vaginal position is at least parallel to the inseminator position.The head of the goat is at the bottom clamped between the legs of the fixator. The implementation of IB is thought to stress the goats. Stress was exacerbated by inserting a speculum to open the vagina so that the hole of the cervical was found as a place for penetration of the inseminator gun.

Stress due to stress causes physiological changes that have an impact on production and reproduction (Ribeiro, 2016). Stress activates the Sympathetic Nerve System whichregulates heart rate and the release of catecholamines and the Hypothalamic Pituitary Adrenal (HPA) axis which regulatesthe release of corticosteroids from the adrenal glands (Stojanović et al. 2012). Reproduction and fertility are regulated through the hypothalamic-pituitary-gonadal hormone (HPG). Stress affects reproductive hormones(Aoyama, 2013). The stress response and the effects of chronic stress involve many molecules in the body responding. This response involves mediators of enzymatic, non-enzymatic, immune, and endocrine molecules

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(Sarangi, 2018). Stressors cause an increase in metabolic processes in the body which causes heat stress in the cells. Heat stress is one of the factors that can cause oxidative stress in vivo. It ischaracterized by an increase in Reactive Oxygen Species (ROS). If the level of ROS in the cell increases than normal, it will trigger damage to polyunsaturated Fatty Acid (PUFA)so that it will damage cell membranes due to increased Malondialdehyde (MDA). In the body, there are enzymatic antioxidants that function as scavengers, namely superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) which act as scavengers both intracellular and extracellular superoxide radicals and prevent lipid peroxidation in plasma membranes (Mellado, 2013). Stress also elicits an inflammatory response with the production of ROS by phagocytic immune cells (Deters and Hansen 2020).The aim of the study was to measure the AI stress response and evaluate the success of intracervical goat AI in smallholder farms.

II. Materials And Method

A. Experimental Animals

The research was conducted at Goat Breeding Station Singosari Malang, East Java, Indonesia. Sample preparation was carried out at the Laboratory of Physiology and Livestock Reproduction, Faculty of Animal Husbandry, University of Islam Malang. Crossbred Ettawa goats as experimental animals were selected according to the criteria: good reproductive history, i.e. at least once giving birth, normal estrus cycle with at least two cycles detected, not pregnant, Body Condition Score 3. Goats were treated with intracervical AI for the first time and natural mating (NA) ascontrols. AI is carried out like AI in smallholder farms. There were 15 goats for each treatment.

B. Adaptation Period

This adaptation was carried out for 14 days. The adaptation period was carried out by measuring the physiological status of goats and weighing body weight.Basal feed is forage (Taiwan Elephant Grass) given 4-5 kg/day and concentrate (polar, DDGS, minerals, copra, and corn) as much as 500 gr/goat/day. Drinking water is providedad libitum.

C. Measurement of Physiological Status

The physiological status variables measured were rectal temperature, respiration and pulses rate as well as weighing body weight (Rosita, 2015). Rectal temperature was measured at the same time as respiration and pulses rate measurements. Measurements were taken three times a day after feeding. Calculations in duplicate using a counter and a stopwatch. Measurement of pulse using a stethoscope. pulse rate is calculated for one minute. All measurements were performed in Duplo.

D. Implementation of Artificial Insemination and Natural Mating

Goats were synchronized with Dinoprost trometamol (luteolytic prostaglandin, Sanbe) before treatment. Synchronization procedure according to Inonu, 2014. Hormone injection is done twice on the first day and the 12th.The dose is 1.5 ml/IM. Both NM and AI are carried out on the 14-15th day. The straw dose for AI is $100x10^6$ spermatozoa. The implementation of goat AI is carried out with reference to the implementation of AI which is often carried out on smallholder farms by Intracervical AI (Inonu, 2014).

E. Ethical Approval

The study procedure was done according to the operational standard (SNI ISO 9001 : 2015 No. G.01- ID.0139-VIII-2019) and controlled by a veterinarian.

F. Blood Plasma Collection

Blood plasma collection for stress molecule examination was carried out a week before and after mating (Romero et al. 2015). Blood as an examination sample was obtained from the jugular vein. Blood collection using venoject with a vacuum tube with EDTA. 5 ml of blood obtained from the jugular vein was left at room temperature for 30 minutes and then 3000 g was centrifuged for 15 minutes. The results of the centrifuge were taken with a micropipette and put in an Eppendorf tube. Store in refrigerator until examination.

G. Stress molecule measurement

The examination of SOD activity was carried out according to Wolfe, (2007). Absorbance readings at a wavelength of 450 nm using a microplate reader. Measurement of catalase enzyme activity according to Hamid(2010). A regression curve for the standard solution of the CAT Assay Kit Biovision was made. Determination of Glutathione Peroxidase (GPx) activity according to Wolfe (2007). Absorbance with a spectrophotometer at a wavelength of 340 nm. Determination of Malondialdehyde (MDA) and preparation of blood samples was carried out according to Singh (2002). Serum/plasma absorbance was measured at 532 nm. As a standard solution used Tetraethoxypropane solution or standard MDA Assay Kit. Neutrophil examination using a Haematology analyzer according to the procedure in the Humacount 30TS user manual

H. Progesterone test

Measurement of progesterone levels using Goat Progesterone Elisa Kit CSB-E13631G. The implementation procedure is in accordance with the product instructions and Roberts, et al (2017). Serum was taken on the 25th day after the mating.

I. Evaluation of Natural Mating and AI Success

Evaluation of the success of natural mating and AI-basedon parameters: Conception rate, Birth weight, Litter size, and weaning weight. Weaning weight was measured in 90 days old goats (Kaunang et al. 2014).

J. Statistical Analysis

The statistical was done using the IBM SPSS 25.0 window. The difference of type was obtained using thestudent t-test and $P<0.05$ was considered statistically significant.

III. Result

A. Physiological Status before and after mating

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The results of the paired t-test showed that the measurement of the physiological status of goats, namely rectal temperature before and after mating, both natural mating and artificial insemination did not different (p>0.05) while pulse and respiratory rate before and after mating weredifferent (p<0.05).

TABLE 1. Differences in physiological status between before and after of natural mating and artificial insemination of Crossbred Ettawah Goats

Parameter	NA		AI		Note
	Before	After	Before	After	
Rectal temperatures (^{0}C)	39.02 ± 0.28	39.60 ± 0.30	39.50 ± 0,30	39.70 ± 0,31	
Respiratory rates (minutes)	33.20 ± 0.86	51.00 ± 0.99	30.00 ± 0.78	51.5 ±1.09	*
Pulse rates (minutes)	80.11±4 .45	128.03± 6.05	85.15 ±5.05	130.09±9 .11	*

* = It was significantly different (P<0.05) between before and after of Natural mating or artificial insemination .
NA = Natural Mating
AI = Artificial Insemination

In NM the average rectal temperature before and after matingwas 39.02 ± 0.280C and 39.60 ± 0.300C, while in AI it was 39.50 ± 0.300C and 39.7 ± 0.310C. The average respiratory frequency after mating was higher than before mating. This occurs in the type of NM and AI. The average respiration rateper minute before NM was 33.20 ± 0.86 and after 51.00 ±0.99. In AI the respiratory rate before AI was 30.0 ± 0.78 andafter AI was 51.5 ± 1.09. The average pulse rate after matingis higher than before mating. This occurs in the type of NM and AI. The average pulse per minute before NM was 33.20± 0.86 and after mating 51.00 ± 0.99. In AI the pulse rate before AI is 30.0±0.78 and after AI is 51.5±1.09 (table 1)

B. Stress molecular response between before and after of mating treatment

The results of the t-test showed that the activities of SOD, CAT, GPx, MDA, and neutrophils of goats before and after mating were different (p<0.05) in both NM and AI. The activity of SOD, CAT, GPx, and MDA after mating was higher than before mating. The average SOD activity (unit/200µL) in the NM group before mating was 35.37 ±1.14 and after mating 45.35 ± 1.68 while in the AI group before mating the SOD level was 35.33 ± 1.09 and after mating 52.12 ±2.95.

TABLE 2. The differences of stress molecular response between before and after of mating treatment

Stress molecular responses	NA		AI		Note
	before	After	before	after	
SOD (Unit/200µL)	35.37±1.14	45.35± 1.68	35.33±1.09	53.12±2.95	*
CAT (Unit/200uL)	16.94±0.24	18.542±1.36	17.04±1.18	26.47±1.32	*
GPx (mU/mL)	20.43±0.76	30.47± 2.47	20.19±1.61	38.70±2.40	*
MDA (ng/200 µL)	0.18± 0.01	0.21± 0.02	0.24± 0.01	0.25± 0.02	*
NETROFIL(%)	37.40±2.51	47.40± 1.51	37.00±1.87	58.00±2.55	*

* = It was significantly different (P<0.05) between before and after of Natural mating or artificial insemination .
NA = Natural Mating
AI = Artificial Insemination

CAT activity before mating was lower than after mating in both NM and AI. The average CAT activity (unit/200µL) before and after mating in the NM group was 16.94±0.24 and 18.542±1.36, while in AI it was 17.04±1.18 and 26.47±1.32. GPx activity before mating was lower than after mating in both NM and AI. The average GPx activity (mU/mL) before after mating in the NM group was 20.43±0.76 and 30.47±2.47 and the AI group was 20.19±1.61 and 38.70±2.40, respectively. The MDA levels in both NM and AI groups were higher after mating than before. MDA levels(ng/200µL) before mating in NM groups were 0.18±0.01 while after mating 0.21±0.02. In group AI before mating 0.24± 0.01 and after mating 0.25 ± 0.02. The percentage of neutrophils in both NM and AI groups was higher after mating than before. The percentage of neutrophils in the NMgroup before mating was 37.40±2.51 and after 47.40±1.51 while in the AI group before mating 37.00±1.87 and after 58.00±2.55 (table 2).

C. Measurement of mating success

The measurement of mating success was based on progesterone levels, conception rate, birth weight, litter size and weaning weight. The measurement results for natural mating types, progesterone levels and litter size were different (P<0.05) with IA.

TABLE 3. The Average Measurement from the Variable Indicator of the success of mating

Mating Type	Prog (ng/ml)	CR (%)	BW (kg)	LS (kids/birth) n=15	WW (kg)
NM	10,82±0,35*	100	2,93±0,03	1,6±0,04*	11,06±0,44
IB	4,32±0,05*	20	2,1±0,01	1±0,01*	9,9±0,39

* = It was significantly different (P<0.05) between Natural mating or artificial insemination

Note :
NM = Natural Mating
AI = Artificial Insemination
Prog = Progesterone
CR = Conception rate
LS = Litter size
BW = Birth Weight
WW = Weaning Weight

Progesterone in NM were higher than in AI. Progesterone (ng/ml) in NM were 10.82 ± 0.35, while in AI it was 4.32 ±0.05. Litter size in NM is higher in AI. Littersize of NM 1.6±0.04 while in AI 1±0.01. Birth weight and weaningweight of NM cows did not different (P>0.05) with AI. Birthweight and weaning weight in NM were 2.93±0.03 and 11.06±0.44 while in AI were 2.1±0.01 and 9.9±0.39. Conception rate of NM is 100% and AI is 20% (table 3).

IV. DISCUSSION

Livestock physiological status describes the healthcondition of livestock. This condition depends on the environmental conditions of the livestock (Ghalem et al. 2012). The type of mating did not affect the rectal temperature. The rectal temperature of PE goats before mating did not differ after mating in either the NM type or the first AI. The body temperature is not different because the feed given is the same. Rosita (2015) stated that the rectal temperature of goats ranged

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from 38.50C - 400C. Rectal temperature is influenced by several factors: environmental temperature, livestock activity, feed, drink, and digestion (Gupta, 2013). The frequency of respiration before mating was different from that after mating in both NM and AI. There is a tendency to increase the frequency of respiration after mating, namely in NM 51.00 ± 0.99 times/minute while in AI 51.5 ± 1.09 times/minute. The position of the goat at the time of mating increases the frequency of respiration due to muscle activity during the mating process. This makes energy demands increase. Energy metabolism of course requires oxygen, an increase in oxygen makes respiration increase. The normal frequency of goat respiration accordingto Marai (2007) is 26-54 times/minute.

Heart rate before mating is different from after mating in both NM and AI. There is a tendency to increase heart rate after mating, namely in NM 128.03±.6.05 times/minute whilein AI 130.09±9.11 times/minute. Increased heart rate is still within the normal range. The normal frequency of the pulse rate is 70-135 beats/minute (Marai et al. 2007). Mating both NM and AI resulted in an increase in pulse rate and respiration of goats. This is due to an increase in muscle movement and activity Adelodun (2012). The implementation of mating makes the goat's muscle activity and adrenaline increase so that the heart's performance also increases.

The molecular response to mating-type stress was demonstrated in SOD, CAT, GPx, MDA, and neutrophils. There was a tendency for the molecular stress response to increase in both NM and AI. The increase in stress response was higher in the first AI than in the NM. Intracervical AI with gun and speculum induces an acute-phase cell response. This response is a complex systemic innate defense system and is activated by trauma, infection, inflammation, and stress to prevent tissue damage, eliminate infective organismsand restore homeostasis (Sarangi, 2018). The first experienceof getting AI makes muscle cell activity and metabolism increase. This is followed by an increase in the body's physiological response. Increased cell activity due to IBmakes the production of ROS increase followed by an increase in antioxidants to prevent cell damage to lipids, proteins, and DNA. The body's defense system through the antioxidant system is the initial barrier against cell damage. The antioxidant system formed due to cell stress can be in theform of SOD, GPx, Catalase, and metal groups (Bucak, 2009). Kaushik et al (2018) reported that in Jamunapari goatssubjected to heat stress, the tolerant response to SOD activity was 29.56±3.52%. Meanwhile, the susceptibility to heat stress SOD activity was 28.76±3.67%. CAT activity in the semen of Angora Goats under freezing stress was 6.8 ± 0.9 kU/g prot (Bucak, 2009). The AI treatment for the first time made the goat's respiration increase along with increased muscle activity. Excessive oxidative reactions result in cell functional disorders. Oxidative reactions produce ROS (Valko, 2006). If the amount is excessive, for example, due to respiratory and muscle activity, it causes biological damage known as oxidative stress. Excessive ROS will damage cellular lipids, proteins, and DNA. If the level of ROS in the cell increases than normal, it will trigger the destruction of polyunsaturated Fatty Acid so that it will damage cell membranes due to increased Malondialdehyde (MDA) (Kohen and Nyska., 2002). MDA levels in the semenof Angora Goats subjected to freezing stress of 0.6±0.2 nmol/l were reported by Bucak (2009). The increase in neutrophils in AI was first due to stress which resulted in an increase in endogenous ROS (Marciniak et al. 2009). Stress and muscle activity as an intracervical AI response make white blood cells mobilize from the bone marrow and are distributed in the circulation to the spleen (Engler et al., 2004). Goats under heat stress for 8 neutrophil levels were 30.3 ± 1.5&. The normal value for goat neutrophils is 30-48%(Ribeiro, 2018).

The results of the evaluation of the success of mating found that the levels of progesterone and litter size were different between NM and the first AI. Progesterone levels inNM were 10.82±0.35 ng/ml while AI was 4.32±0.05 ng/ml. Litter size of NM 1,6±0.04 kids/birth and AI 1±0.01 kids/birth. The conception rate of NM is 100% while AI is 20%. The success of a mating is related to reproductive hormones. Stress due to intracervical IB results in an increasein free radicals. This is known from the increased activity ofSOD, CAT, GPx, MDA, and neutrophils. According to Khan(2020), an increase in free radicals results in an increase in cortisol produced by the adrenal cortex, thereby stimulating the production of PGF2α. PGF2 causes lysis of the corpus luteum accompanied by a decrease in the hormone progesterone. Low progesterone is associated with low birth weight and weaning weight. Progesterone plays a role in stimulating the growth and development of the mammary glands in order to prepare a food source (milk production) forthe kid to be born. Progesterone helps increase maternal blood flow and growth of the chorionic villi (Kalantaridou, 2010).

V. CONCLUSION

Research results show that intracervical AI in smallholder farms causes stress in goats. Stress response was measured by SOD, CAT, GPx, MDA, and Neutrophils. The molecular stress response before and after mating differed between natural mating and IB first. The activities of SOD, CAT, GPx,MDA, and Neutrophils in AI were higher than in natural mating. The results of evaluation of the success of mating showed that the level of progesterone, conception rate, birth weight, litter size, and weaning weight in natural mating washigher than AI.

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