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Original article

Intracervical artificial insemination using frozen semen of Boer buck on Pote does at different times of estrus

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ABSTRACT

Pote goats are kept by the rural community in Bangkalan regency, Madura island, Indonesia. Symptoms of estrus in goats are usually unclear, causing low fertility. This study aims to evaluate intracervical artificial insemination performed in early and mid-estrus on Pote does. Twenty four does were randomly divided into two groups and their estrous was synchronized using two injections of 200 µg PGF2α intramuscularly with 11 days interval. In the T1 group, Pote does that were in estrus were inseminated at early estrus (0-12 hours after signs of estrus appeared), while in the T2 group, Pote does that were in estrus were inseminated at mid-estrus (12-24 hours after signs of estrus appeared). The insemination conducted artificially using frozen-thawed Boer semen. The observed variables were non-return rate at day-21 post-insemination (NRR21), and pregnancy rate (PR) based on abdominal palpation diagnosis. The result showed that frozen-thawed Boer semen was qualified (spermatozoa motility more than 40%) for artificial insemination. All Pote does were in estrus after two intramuscular injection of PGF2α with an interval of 11 days. The onset of estrus was 48-72 hours after the second injection PGF2α. The NRR21 and PR of the T2 group (66.7 and 100%) were higher than those of the T1 group (37.5 and 66.7%), respectively. It could be concluded that intracervical artificial insemination in Pote does in mid-estrus was more effective, resulting in higher NRR21 and PR than in early estrus.

Keywords: artificial insemination, estrus phase, non-return rate, Pote does, pregnancy rate

INTRODUCTION

Pote goats are dual-purpose goats i.e., meat goats, and milk producers. Pote goats are kept by the rural community in Bangkalan Regency, Madura Island, Indonesia. The fur color of the Pote goat is solid white (Pote comes from the Madurese language, which means white). The body posture of the Pote goat is smaller than the other goat variants. The ears are medium in size, longer than the ears of the Kacang goat, but not as long as the ears of the Ettawa breed; the lips are pink, the tail is protruding, and the males and females have beards. The adult body weight of the male Pote goat is 25-40 kg, and the female is 20-35 kg. There are no scientific publications

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about Pote goat yet. In addition, Pote goats had never been artificial inseminated. Artificial insemination is expected to increase the population and spread the distribution of Pote goats to other areas of Madura. The advantage of the Artificial Insemination program in goats was that they could give birth three times within two years to accelerate the increase in the goat population (Luo *et al*., 2019).

Lack of estrus detection was one of the factors caused low pregnancy rates of Artificial Insemination implementation (Chanda *et al*., 2020). Therefore, recognizing the estrus period of does was one of the determinants for farmers to gain profits from the goat. Detection of estrus by the owner of the goats himself was necessary because it was the breeder who had daily contact with his goats. Farmers' skill in detecting their does' estrus influenced the success of artificial insemination (Rahmah *et al*., 2018).

The estrus synchronization with PGF 2α could facilitate estrus detection (Holtz *et al*., 2008; Martemucci and D'Alessandro, 2011) and overcame the low mating efficiency (Goodling *et al*., 2005). The effectiveness of estrous synchronization could be assessed through symptoms of sexual behavior, and the pregnancy result (Isabel *et al*., 2018). Estrus symptoms in goats were usually unclear (Simoes, 2016). Symptoms of estrus in goats that are often seen are restlessness, bleating, riding on other goats, and rubbing their bodies against the walls of the barn. A perfect estrus of goats showed obvious clinical symptoms such as vulva enlargement, swelling, and warm and mucus discharge (Salleh *et al*., 2021). Therefore, this study aims to determine the non-return rates on day 21 after insemination (NRR21) and the pregnancy rates (PR) after intracervical artificial insemination at early and mid-estrus (respectively 0-12 hours, and 12-24 hours after signs of estrus appeared).

MATERIALS AND METHODS

This study was conducted at Alaskokon village, Modung district, Bangkalan regency, East Java, Indonesia. This study site is located at coordinates of 12°40'06"–113°08'04" East longitude and 6°51'39"–7°11'39" South latitude with an altitude of 5m above sea level. Climate and average weather throughout the year were generally hot; temperatures varied from 22-34 °C, and relative humidity was 68-83%. Annual rainfall ranges from 1,200-1,800 mm, with the number of rainy days being 80-120 days per year (Adisiswanto, 2020).

This study used 24 Pote does aged 2-4 years, weighing 25-30 kg, in good health, and not pregnant based on abdominal palpation examination. Feeding consisted of forage of 4-5 kg, concentrate of 0.5 kg/day, and water provided at any time. The semen used for artificial insemination was frozen semen of Boer buck produced by the Ungaran Artificial Insemination Center, Semarang, Central Java, Indonesia.

Treatment of the does

Twenty four Pote does were randomly divided into two groups. Estrous synchronization was performed using 200 µg PGF2α (Cloprochem) which was injected twice intramuscularly with an interval of 11 days (Raymundo *et al*., 2021). The onset of estrus (in hours) was determined based on the interval from the last PGF2α injection to the first estrus symptom (restlessness, riding on other goat, and rubbing their bodies against the housing wall). The perfect estrus is marked by a reddish, swollen, warm vulva and discharge of watery mucus from the vagina (Triagil *et al*., 2020). Data of the onset of estrus were evaluated using independent t-test in Statistical Product and Service Solution (SPSS) version 23 for Windows at 5% level of significance. Group T1 was Pote does which were in estrus, and inseminated at the beginning of estrus (0-12 hours after estrus symptoms appeared), while Group T2 was Pote does which were in estrus, and were inseminated in mid-estrus (12-24 hours after symptoms of estrus appeared). Artificial insemination was conducted intra-cervically using All-2-Mate® goat insemination gun guided by a speculum.

Frozen-thawed semen evaluation

Thawing of Boer buck frozen semen was conducted at 37^{o}C for 30 seconds. Semen quality was evaluated based on progressive spermatozoa

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motility, viability, and intact plasma membrane. Spermatozoa motility assessment was conducted by dripping10 µL of thawed semen onto an object glass and then covering it. Progressive spermatozoa motility was counted on 100 spermatozoa under a phase contrast microscope (Olympus BX TF, Japan) with 400x magnification (Susilowati *et al*., 2021). Spermatozoa viability assessment was conducted by dripping a drop of frozen-thawed semen onto an object glass, added with eosin nigrosine solution (Merck Germany), homogenized, smeared, and fixed quickly over a flame. The slide was examined under a microscope (Olympus BX TF, Japan) with a magnification of 400x for 3-5 fields of view to reach 100 spermatozoa. Reddish-head spermatozoa indicated the dead spermatozoa, and colorless (translucent) spermatozoa indicated the live spermatozoa (Susilowati *et al*., 2020). The assessment of plasma membrane integrity was conducted by mixing 0.1 mL of frozen-thawed semen with 1 mL of Hypoosmotic solution. The mixture was then incubated at 37°C for 60 minutes, and examined under a microscope (Olympus BX TF, Japan) with 400x magnification for 100 spermatozoa. The coiled of the spermatozoa tail indicated that the plasma membrane of the spermatozoa was still intact. Meanwhile, a straight form of the spermatozoa tail indicated damage to the spermatozoa plasma membrane (Susilowati *et al*., 2021).

The artificial insemination result was evaluated based on the non-return rates of estrus 21 days after AI (NRR21) (Al'A'raaf *et al*., 2020). Sixty days after artificial insemination, a pregnancy examination was conducted using the abdominal palpation method (Karadaev, 2015). The data on frozen-thawed semen quality, estrus rates, onset of estrus, NRR21 and PR are presented descriptively.

RESULTS

Estrus response of Pote does after PGF2α injection showed that all Pote does were in estrus (Table 1).

Table 1 Estrus response of Pote does after PGF2α injection

	estrus rate	onset of estrus (hours)
T1	100%	48 ± 1.54 [b]
T2	100%	72 ± 1.93 [a]

Different superscripts in same column indicated significant difference ($p < 0.05$); T1: the Pote does which were in estrus, were inseminated early in estrus (0-12 hours after estrus symptoms appeared); T2: the Pote does which were in estrus, were inseminated in mid-estrus (12-24 hours after symptoms of estrus appeared); n: 12 does.

Assessment of frozen-thawed Boer buck semen (Table 2) indicated spermatozoa motility of more than 40%, which met the quality requirement for artificial insemination. The NRR21 and PR of Pote does inseminated in mid-estrus were higher than those of inseminated in early estrus (Table 3).

Table 2 Quality of post-thawed semen of Boer buck

	average (%)
spermatozoa motility	40 ± 0.52
spermatozoa viability	44 ± 0.56
intact plasma membrane	42 ± 0.43

Table 3 Evaluation of Pote does after artificial insemination

	non-return rate	pregnancy rates
T1	66.7% (8/12)	37.5% (3/8)
T2	100.0% (12/12)	66.7% (8/12)

T1: the Pote does which were in estrus, were inseminated early in estrus (0-12 hours after estrus symptoms appeared). T2: the Pote does which were in estrus, were inseminated in mid-estrus (12-24 hours after signs of estrus appeared).

DISCUSSION

Estrus synchronization is expected so that the does were in estrus simultaneously and kidding at the same average time (Pangestuningrum *et al*., 2021). Prostaglandins

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F2α could be injected intramuscularly for estrus synchronization in does. Prostaglandin F2α is the luteolytic agent in ruminants; therefore, prostaglandins should be administered in the luteal phase. Luteolysis will induce the subsequent follicular phase of the estrous cycle, leading to ovulation. Two injections of prostaglandin F2α administered 9-11 days apart resulted in a higher estrus response (92.8%) than a single treatment (75%) in the Red Sokoto does (Omontese, 2018). In this study, all Pote does were in estrus after two PGF2α intramuscular injection with an interval of 11 days. The onset of estrus was 48-72 hours after the second injection (Table 1). These results were within the range of 36-72 hours after the last PGF2α intramuscular injection reported by Budiyanto and co-workers (2020). However, this result was longer than that of Bulgarian White does with natural and synchronized estrus occurring between 24-48 hours (Yotov *et al*., 2016).

The onset of estrus of does inseminated in mid-estrus (72 ± 1.93 hours) was higher than those inseminated earlier (48 ± 1.54 hours). This result was similar to estrus synchronization in Ongole cross using intramuscular PGF2α alone and combined with GnRH 24 hours later which achieved onset of estrus in 75.9 ± 0.8 and 41.6 ± 28.4 hours respectively (Astuti *et al*., 2020). PGF2α injection at the end of estrus cycle induced CL regression than those at early luteal phase (Hajibemani *et al*., 2022). On PGF2α injection in the early luteal phase, it is possible that CL was not mature enough to respond to luteolysis (Levy *et al*., 2000). Unfortunately, in this study the detection of the luteal phase was not conducted using ultrasound; therefore, it was not known whether the CL was in the early or late luteal phase.

Injection of PGF2α for estrous synchronization on several does caused shortening of the luteal phase by lysing the corpus luteum of the does simultaneously within 2-3 days (Al Yacoub *et al*., 2011). Injection of PGF2α would cause a constriction of the utero-ovarian blood vessels resulting in lutein cells hypoxia and lysis followed by a decrease in progesterone levels (Carlos *et al*., 2019). The basal level of progesterone led to the disappearance of negative feedback on the hypothalamic-pituitary axis, stimulating the release of Follicle Stimulating Hormone (FSH) and LH. Pulsatile of FSH and LH were responsible for folliculogenesis, synthesis, and excretion of estrogen for the symptoms of estrus, and eventually, the LH surge induced ovulation (Nogueira *et al*., 2015).

The motility of frozen-thawed Boer buck semen was more than 40%, meeting the requirement for artificial insemination (INSA, 2014). In this study, intracervical artificial insemination of the estrus does resulted in non-return rates, and pregnancy rates ranging from 66.7-100% and 37.5-66.7%, respectively. As a comparison, the non-return rate of Boer goats was 73.3-93.3% (Salim *et al*., 2020), and the pregnancy rate for local goat was 75% (Budiyanto *et al*., 2020). Another study reported that the pregnancy rate after hormonal treatment was 62.5% in Payoya goats during the anestrous season (Arrebola *et al*., 2022). The non-return rate is the proportion of the does that are not subsequently re-bred within a specified period after insemination. Meanwhile, the pregnancy rate is the proportion of the does that are pregnant based on accurate methods of pregnancy diagnosis (Yotov *et al*., 2016). Transabdominal palpation is cheap and easy to do in field conditions, with more than 90% accuracy after 60 days of mating (Memon and Ott, 1980).

The does that did not return to estrus after insemination was not necessarily pregnant. The pregnancy rate was lower than the non-return rate in both the does inseminated in early and mid-estrus, which could be due to early embryonic death. After fertilization, zygote would develop to be a blastocyst. The trophectoderm of the blastocyst produced interferon tau in 2–4 weeks after conception as maternal recognition of pregnancy (Bazer and Thatcher, 2017). The paracrine effect of interferon tau on the endometrial epithelium inhibited the upregulation of oxytocin receptors, prevented PGFα excretion followed by the prevention of luteolysis, and maintained progesterone at high level for a few weeks (Hansen *et al*., 2017). The early embryonic

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deaths during the peri-implantation period (Bazer and Thatcher, 2017) occurred in the non-return to estrus (Karadaev, 2015) with high levels of progesterone at 25–30 days post-mating (Islam *et al*., 2014).

The NRR21 and pregnancy rate values for does inseminated in mid-estrus were higher than those inseminated earlier, possibly because the higher percentage of the does ovulating at the time of insemination. Optimal fertility was obtained when semen deposited in the inner cervical lumen of the estrus does (Omontese, 2018). Higher NRR21 and PR were achieved when the does were inseminated closest to the time of ovulation (Leigh *et al*., 2018; Riaz *et al*., 2018). After being deposited into the cervical canal, spermatozoa would undergo capacitation and have to migrate to the site of fertilization (Gervasi and Visconti, 2016). Fertilization occured in the ampullary-isthmic junction and required the proper timing of insemination and ovulation. Spermatozoa remained viable for only 12 hours in the female reproductive tract, and the life span of the ovulated egg was limited to 12-24 hours (Yanagimachi, 2022). Estrus in goats lasted around 24-48 hours, in an average of 36 hours (Dávila *et al*., 2018). Ovulation could occur 9–72 hours after the onset of estrus. Subsequently, around 48 h after the estrogen's peak, a sudden LH surge occurs, which promoted ovulation 20–26 h later (Arrebola *et al*., 2022). Therefore, intracervical artificial insemination in the mid-estrus of Pote does resulted in a higher NRR21 and pregnancy rate than those at early estrus.

CONCLUSION

Intracervical artificial insemination of Pote does performed in mid-estrus was more effective than in early estrus.

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AUTHORS' CONTRIBUTIONS

Suherni Susilowati (SS), Tri Wahyu Suprayogi (TWS), Tatik Hernawati (TH), Yossy Imam Candika (YIC), Widya Paramita Lokapirnasari (WPL)

SS and TWS conceived the idea and designed the mainframe of this manuscript. SS, TH, and YIC collected data. SS and WPL analyze data and drafted the manuscript. TWS, TH, YIC critically read and revised the manuscript for its intellectual content. All of the authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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