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Effects of Different GnRH Doses Combined with PGF2α on Estrus Characteristics and Artificial Insemination Success in Thin-Tailed Sheep

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ABSTRACT

Estrus synchronization is one method to standardize the estrus cycle in thin-tailed sheep. The objective of this study was to evaluate estrus quality and the success of artificial insemination using PGF2α hormone combined with GnRH. Hormones were administered by intramuscular (IM) injection. Sixteen female thin-tailed sheep aged 2–3 years with body weights of 33–35 kg were used. Treatments were divided into four groups with four replications: P0 (PGF2α 1 ml + GnRH 0 ml), P1 (PGF2α 1 ml + GnRH 0.5 ml), P2 (PGF2α 1 ml + GnRH 1 ml), and P3 (PGF2α 1 ml + GnRH 1.5 ml). Estrus observation was conducted visually. Artificial insemination was performed using frozen semen, and pregnancy detection was carried out with the PregnaDrop test kit. Data were analyzed using descriptive statistics and the Kruskal–Wallis test. The results showed that the administration of GnRH and PGF2α significantly affected the success of artificial insemination, with estrus onset differing significantly ($P<0.05$) and estrus intensity differing highly significantly ($P<0.05$). The best dose was found in P3 (PGF2α 1 ml + GnRH 1.5 ml).

Keywords: Dosing, Artificial Insemination, GnRH, PGF2α, and Synchronization

INTRODUCTION

The increasing demand for animal protein requires local farmers to meet community needs through effective livestock management, employing various strategies to ensure a sufficient supply. One of the most effective ways to increase the population of thin-tailed sheep is through improved breeding management (Astuti et al., 2019). Breeding can be carried out using two methods: natural mating and artificial insemination (AI). AI is considered an ideal method for accelerating livestock population growth. Before implementing either natural mating or AI, accurate detection of estrus in female sheep is essential. Estrus is characterized by vulvar redness and swelling, increased temperature, cervical mucus secretion, and behavioral changes (Udin et al., 2016).

Estrus synchronization is a reproductive technique designed to manipulate the estrus cycle so that ovulation and estrus signs occur simultaneously (Efendi et al., 2015). The administration of PGF2α is a synchronization method used in sheep. Hormonal administration via injection of PGF2α and GnRH directly into or near the target organ improves efficacy, reduces the required dose, and minimizes costs. The use of GnRH in synchronization protocols aims to stimulate follicular development and ovulation. The use of GnRH in synchronization protocols aims to stimulate follicular development and induce ovulation. PGF2α, on the other hand, promotes corpus luteum regression, which decreases progesterone levels and facilitates the subsequent ovulatory response. When combined, GnRH and PGF2α improve estrus quality and fertility. Previous studies in goats demonstrated that GnRH administration increased estrus response and conception rates (Yendraliza, et al., 2020). Similarly, Efendi et al., (2015) Reported that GnRH injection in sheep induced accessory corpus luteum formation, which enhanced reproductive performance.

Therefore, this study was conducted to evaluate the effect of different doses of GnRH combined with PGF2α on estrus characteristics and the success of artificial insemination in thin-tailed sheep. The findings are expected to provide practical recommendations for optimizing estrus synchronization protocols in small ruminants,

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thereby supporting livestock productivity, and meeting the growing demand for animal protein.

MATERIALS AND METHODS

The experimental animals consisted of 16 female thin-tailed sheep aged 2–3 years, weighing 33–35 kg, healthy, and not pregnant. Equipment used included 3 ml and 1 ml syringes, GnRH, PGF2α, frozen sheep semen, artificial insemination gun, scissors, and vaginoscope.

Research Variables

1. Estrus Intensity

Estrus intensity was assessed based on physiological and behavioral signs, including vulvar redness and swelling, mucus secretion, and behavioral changes such as tail lifting and vocalization. The scoring system followed Santoso et al., (2014), ranging from 1 (no signs) to 4 (clear signs).

2. Estrus Onset

Estrus onset was defined as the time required for sheep to exhibit estrus signs after the final hormone injection. (Ilham et al., 2016).

3. Artificial Insemination Success

Artificial Insemination success was determined by detecting pregnancy with the PregnaDrop test kit, which identifies changes in urine color and sedimentation. (Yendraliza, et al., 2020).

Research Procedure

Selected sheep were injected intramuscularly with PGF2α, followed by GnRH according to treatment groups. Estrus signs were observed visually, and artificial insemination was performed using frozen semen approximately 12–18 hours after the onset of estrus. Pregnancy was checked on day 18 post-insemination using the PregnaDrop kit (Yendraliza, et al., 2020).

Data Analysis

Data were analyzed using descriptive statistics to summarize the observed variables. Statistical analysis was performed using the Kruskal–Wallis test to determine differences among treatments. (Udin et al., 2016). When significant differences were found ($p < 0.05$), pairwise comparisons were conducted using the Mann–Whitney U test to identify which treatment groups differed.

Table 1. Estrus Intensity Scoring Criteria

No	Assessment Criteria	Score	Description
1	Vulvar Changes	4	The swollen vulva appears red
		3	Moderately swollen, reddish vulva
		2	Slight swelling, vulva turns pink
		1	No swelling, pale vulva
2	Mucus	4	Abundant mucus discharge
		3	Mucus around the vulva only
		2	Mucus is visible upon the vulva opening
		1	No mucus
3	Behavior	4	Tail lifted, stands still when mounted, and has frequent bleating
		3	Tail flicking, tail covers when mounted, and stands still.
		2	Avoids mounting, restless, tail flicking
		1	Refuses to be mounted

Source: Santoso et al. (2014)

RESULTS AND DISCUSSION

The administration of GnRH combined with PGF2α showed an evident influence on estrus characteristics and reproductive performance in thin-tailed sheep. Observations included the onset of estrus, estrus intensity scores, and pregnancy success rates across treatment groups. Statistical analysis revealed significant differences in estrus onset ($P<0.05$) and highly significant differences in estrus intensity ($P<0.05$), while pregnancy success rates varied among treatments. To provide a comprehensive overview, the results of these three parameters are presented together in a single table for clarity and ease of comparison.

Table 1. Result of Estrus Onset, Estrus Intensity Score, and Pregnancy Success Rate

Variable	P0	P1	P2	P3
Estrus Onset (h)	37.51 ± 1.07^{a}	29.56 ± 0.83^{b}	27.81 ± 0.64^{b}	27.67 ± 1.07^{b}
Estrus Intensity Score	5.00 ± 0.81^{a}	8.25 ± 0.95^{b}	9.50 ± 1.00^{b}	11.25 ± 1.50^{b}
Pregnancy Success Rate (%)	50	50	75	75

Different superscripts in the same row indicate significant differences ($P<0.05$).

Estrus Onset

Estrus onset refers to the speed at which estrus symptoms appear, measured as the interval from hormone administration to the emergence of estrus signs. This factor is crucial for improving the success of pregnancy in livestock through artificial insemination. Estrus onset is closely related to the timing of ovulation. Kruskal–Wallis analysis showed a significant difference ($P<0,05$), indicating that different doses of GnRH combined with PGF2α significantly affected estrus onset. The average onset in this study was several hours faster than that reported by Panicker et al. (2015), who found that 1 ml GnRH resulted in an average onset of 49.92 ± 1.94 hours in sheep. The fastest estrus onset was observed in P3 (1.5 ml GnRH), likely due to the higher dose accelerating follicular maturation and inducing ovulation. GnRH stimulates the release of LH, which triggers ovulation (Ilham et al., 2016). Several factors, including age, body condition, and nutritional adequacy, can also influence estrus onset (Susilawati, 2011); seasonal photoperiod (Toelihere, 1993); breed differences; and stress or management practices (Wang et al., 2019). These factors collectively explain variability in estrous responses among individuals, even under similar synchronization protocols.

Estrus Intensity

Estrus intensity reflects the quality of estrus based on the number and clarity of symptoms. The more pronounced the signs, the better the estrus quality. Intensity was scored cumulatively based on vulvar changes, mucus secretion, and behavior. Based on Table 1, the Kruskal–Wallis test revealed a highly significant difference ($p<0.05$), indicating that different doses of GnRH combined with PGF2α significantly influenced estrus intensity in thin-tailed sheep. The highest intensity was observed in treatment P3 (1.5 ml GnRH), suggesting that administration of GnRH 48 hours after PGF2α effectively induces ovulation and enhances follicular maturation, thereby improving fertility. Higher estrus scores reflect better estrus quality, as more apparent signs facilitate more accurate detection and timely artificial insemination. In this study, estrus intensity scores were calculated cumulatively based on vulvar appearance, mucus secretion, and behavioral signs (Abidin et al., 2012).

The estrous response rate in this study was 100%, likely due to hormone administration in reproductively healthy animals and the effectiveness of hormone stimulation targeting specific reproductive organs. According to Udin et al., (2016)Estrus signs include vulvar redness, temperature changes, swelling, mucus discharge, and behavioral changes. One indicator of whether the animal was in estrus after hormone injection was the presence of these signs. Each treatment group exhibited different estrus responses. The best response was observed in P3 (1.5 ml GnRH), where the vulva showed swelling, redness, and abundant mucus secretion. In contrast, animals in P0, P1, and P2 showed only mild mucus discharge. According to Yendraliza et al., (2020) The mechanism of estrus synchronization using the Ovsynch protocol involves administering the first GnRH injection to complete follicular development, ensuring that all animals are in the same follicular stage. Seven days after the initial GnRH injection, PGF2α is administered at a dose of 1 ml. The purpose of PGF2α administration is to induce the corpus luteum (CL) phase, characterized by a decrease in plasma progesterone levels and stimulation of the hypothalamus, which triggers the release of FSH and LH from the pituitary gland. Finally, a second GnRH injection is administered 48 hours after PGF2α administration to synchronize follicular development, thereby enabling simultaneous artificial insemination.

Artificial Insemination Success

Pregnancy success in sheep is influenced by hormonal synchronization, timing of insemination, and accurate pregnancy detection. In this study, pregnancy detection was conducted on the 18th day post-artificial insemination (AI) using the PregnaDrop pregnancy test kit, which identifies pregnancy status based on urine clarity and sedimentation. Clear urine indicates pregnancy, while cloudy urine with sediment suggests non-pregnancy (Yendraliza et al., 2020).

Pregnancy success was defined as the number of pregnant sheep per treatment group. The results showed that groups P2 (1 ml GnRH) and P3 (1.5 ml GnRH) had the highest pregnancy rates at 75%, compared to 50% in P0 and P1. This suggests that higher doses of GnRH enhance follicular development and ovulation, thereby increasing the likelihood of successful fertilization (Panicker et al., 2015)

These findings are consistent with previous studies indicating that GnRH administration improves estrus response and conception rates, especially when combined with PGF2α in synchronization protocols (Santoso et al., 2014). The use of PregnaDrop as a non-invasive, field-friendly diagnostic tool also supports early pregnancy detection, enabling timely reproductive management decisions.

CONCLUSION

Based on the results of this study, it can be concluded that the administration of different doses of GnRH combined with PGF2α significantly affects the success of artificial insemination in thin-tailed sheep. The best dose of GnRH was found in treatment P3 (1.5 ml GnRH), which showed significantly better estrus characteristics compared to P0 (0 ml GnRH), P1 (0.5 ml GnRH), and P2 (1 ml GnRH).

Suggestions

Further studies should not only evaluate the minimal effective doses of GnRH in synchronization protocols to reduce costs while maintaining high estrus quality and AI success, but also investigate the optimal timing of insemination relative to estrus onset to maximize conception rates. Research should explore the influence of nutritional status, body condition, and breed differences on the responsiveness to hormonal treatments, as these factors may contribute to variability in reproductive outcomes. In addition, future work could assess the long-term reproductive performance of ewes subjected to repeated synchronization protocols, including potential impacts on fertility, lambing rates, and animal welfare. Comparative studies between intramuscular and alternative routes of hormone administration may also provide insights into practical applications for smallholder farmers.

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