Application of endoscopic artificial insemination techniques in increasing the crossing dorper sheep population in Indonesia

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Abstract

Endoscopic artificial insemination is an advanced reproductive technique that began to be performed on small ruminants such as sheep by inserting a special instrument into the uterus to inject semen. This research aims to increase the mass population of Indoneian local sheep through endoscopic artificial insemination techniques compared with natural mating techniques and transcervical artificial insemination techniques for locally produced sheep offspring. This research was conducted from June to October 2023 in Taman District, Sidoarjo, East Java, Indonesia, specifically Mendomart Farm. A total of 60 Indonesian local sheep were needed, divided into 3 treatment group. The use of a simple endoscope with a modified trocard using 6 LED 5.5 mm Android Waterproof IPX67 Electronic Lens which is connected to a cellular phone and a modified artificial insemination tool. After treatment in the three groups above, all ewes were checked for pregnancy from day 28 to day 39 post insemination using Dawei vet 6 china intra rectal ultrasonography. The results of this study showed that the average occurrence of oestrus in groups P1, P2, and P3 on day 17 occurred at the same time, namely 55 hours, while the level of oestrus in all treatment groups showed a percentage of 100% (table 1). The pregnancy rate of sheep in each treatment group was 85% after natural mating, transcervical artificial insemination and endoscopic artificial insemination. The use of endoscopic artificial insemination techniques is more efficient than natural mating techniques and transcervical artificial insemination techniques for ewes offspring produced in large populations.

Keywords: *Endoscopic artificial insemination, local sheep, dorper sheep, pregnancy, cement*

Introduction

The decline in the quality of dorper sheep in Indonesia is due to the frequent occurrence of natural mating or inbreeding which is rarely provided with artificial insemination technology and lust synchronization [1]. Therefore, endoscopic artificial insemination techniques can be an alternative in increasing the dorper sheep population. Endoscopic artificial insemination is an advanced reproductive technique that began to be performed on small ruminants such as sheep by inserting a special instrument into the uterus to inject semen [2]. This technique has its own advantages compared to the transcervical artificial insemination technique, namely that the cement inserted can pass through obstacles in the reproductive tract and tortuous cervical rings and allows the deposition of semen in the uterus [3]. Pregnancy rates using fresh, diluted semen deposited in the uterus via endoscopy consistently produce higher pregnancy rates of 60-80% when compared with natural mating techniques and transcervical artificial insemination [4].

In addition, endoscopic artificial insemination techniques require a lower concentration of spermatozoa to be used each time, resulting in a greater number of ewes that can be mated per ejaculation [5]. The average dose required to mate a ewe using endoscopic artificial insemination is less than 20 – 25 million live spermatozoa, when compared with the higher dose required by the natural mating method of ±400 million live spermatozoa and transcervical artificial insemination of 100 – 200 million live spermatozoa [3]. Therefore, through endoscopic artificial insemination it is hoped that every one ejaculation of fresh, diluted dorper semen can be used to inseminate as many as 50 local ewes, resulting in more efficient use of semen. However, the main drawbacks of providing endoscopic artificial insemination services are the high cost of equipment and the relative lack of surgical expertise required to perform this technique safely [6]. However, with the availability of newer, more portable endoscopic equipment, this method can now be offered cost-effectively on farms.

Before performing this technique, it is recommended not to feed and water the animal for 12 – 24 hours to reduce rumen contents and provide better visibility of the reproductive tract. In most cases, sedation using lidocaine hydrochloride is necessary to maintain control of the lamb before and during the endoscopic artificial insemination procedure [7]. This procedure can be performed relatively quickly in 2 to 4 minutes with the animal positioned upside down depending on the skill of the veterinary surgeon. Most animals (>90%) recovered quickly and were able to stand after the endoscopic artificial insemination technique was performed [8]. As with other endoscopic procedures, electrogenic trauma to the abdominal organs which causes acute bleeding, perforation, fecal contamination of the stomach, peritonitis, septicemia and death are possible complications, therefore good surgical skills are needed to avoid these complications [9].

Endoscopic artificial insemination is carried out with the help of a camera endoscopy app program which is created in such a way as to connect an Android camera to the trocard. This technique is carried out by inserting a 26 G long needle coated with a plastic introducer sleeve on the right side of the linea alba (stomach) with the aim of inserting fresh cement directly into the uterine cornua [10]. The important thing to note is the administration of Medroxy Progesterone Acetate (MPA), PGF2α (dinoprost), and PG 600 (a combination of PMSG and Chorulon) to synchronize estrus in sheep [11]. In Indonesia, the technique of endoscopic artificial insemination in sheep has never been used. This research aims to increase the mass population of dorper sheep through endoscopic artificial insemination techniques compared with natural mating techniques and transcervical artificial insemination techniques for locally produced sheep offspring.

Materials and Methods

Ethical approval

The research was approved by animal care and use committee, Universitas Airlangga with No: 1241/HRECC.FODM/XI/2023.

Study area

This research was conducted from June to October 2023 in Taman District, Sidoarjo, East Java, Indonesia, specifically Mendomart Farm.

Inclusion criteria

First of all, the animals were carefully selected, including local Indonesian female sheep that were not pregnant, which were examined using Chinese Dawei Vet 6 ultrasound diagnostics and had previously given birth with a body condition score (BCS) > 2. A total of 60 Indonesian local sheep were needed, divided into 3 treatment group.

Data collection

Group P1 consisted of 20 local sheep receiving 50 mg MPA sponge intra vaginally for 14 days, then after 14 days the sponge was removed and given intramuscularly, PGF2α (dinoprost) 5 mg intramuscularly, and PG 600 (a combination of PMSG and Chorulon) 200 IU intramuscularly. When signs of lust appear on the 17th day in the form of red, swollen, warm genital lips and clear mucus, then natural mating is carried out with 20 male Dorper sheep. The average amount of semen ejaculated by male Dorper sheep is 0.5 cc per ejaculation. diluted with andromed 1: 1.

Group P2 consisted of 20 local sheep receiving 50 mg of MPA sponge intra vaginally for 14 days, then after 14 days the sponge was removed and then given an injection of Equine chorionic gonadotrophin (eCG) 200 IU intra muscularly, PGF2α (dinoprost) 5 mg intra muscularly, and PG 600 (combination of PMSG and Chorulon) 200 IU intramuscularly. When signs of lust appear on the 17th day in the form of red, swollen, warm genital lips and clear mucus, then transcervical artificial insemination is carried out with 0.25 cc of semen with dorper semen dilution ratio of 1:1 with Andromed Germany diluent.

Group P3 consisted of 20 local sheep receiving 50 mg of MPA sponge intra vaginally for 14 days, then after 14 days the sponge was removed and then given an injection of Equine chorionic gonadotrophin (eCG) 200 IU intra muscularly, PGF2α (dinoprost) 5 mg intra muscularly, and PG 600 (combination of PMSG and Chorulon) 200 IU intramuscularly. When signs of lust appear on the 17th day in the form of red, swollen, warm genital lips and clear mucus, then endoscopic artificial insemination is carried out with 0.1 cc of semen with a semen dilution ratio of 1:1 with Andromed Germany diluent.
Endoscopic artificial insemination: The use of a simple endoscope with a modified trocard using 6 LED 5.5 mm Android Waterproof IPX67 Electronic Lens which is connected to a cellular phone and a modified artificial insemination tool is used with the criteria for the length of the trocard introducer cannula being 21 cm, diameter 4 mm, needle length 21cm 26 G (Figure 1).

Figure 1. Endoscopic artificial insemination technique

The female Indonesian local sheep is positioned at a 45° angle hanging upside down and the hind legs are tied. The wool on the abdominal cavity is cut from the mammary glands and extends cranially to the umbilicus. Then the area is rubbed with 70% alcohol. Local analgesia in the form of Lidocaine hydrochloride was administered at each incision site at a dose of 2 ml. A scalpel 11 is used to make a 0.5 inch incision through the skin and fascia to the level of the muscle above the incision site. After the reproductive tract is visible, an endoscopic artificial insemination tool in the form of a trocard containing a 0.1 cc cement cartridge with an outer sheath is then inserted near the nipple cannula and aligned opposite the large curve of the uterine horn under the guidance of an endoscope connected to a cellular cell phone. The Aspic apparatus and needle are then placed aimed as close as possible to the uterine horn and with a quick puncture, the needle is placed at the level of the middle of the uterine horn. Cement can be injected into one or both uterine horns according to the operator's wishes. Avoid inserting the bladder incorrectly because death can occur. After cement injection, the trocard is immediately withdrawn to ensure there is no excessive bleeding or lacerations to the uterine horn.

Pregnancy check

After treatment in the three groups above, all ewes were checked for pregnancy from day 28 to day 39 post insemination using Dawei vet 6 china intra rectal ultrasonography.

Data analysis

All data obtained was analyzed based on the level of lust and success of pregnancy.

Result

The level of difficulty in the application of endoscopic artificial insemination can be solved when researchers have carried out repeated monitoring of intrauterine semen injection using the camera endoscopy app program as a result of equipment modification by researchers. The results of this study showed that the average occurrence of oestrus in groups P1, P2, and P3 on day 17 occurred at the same time, namely 55 hours, while the level of oestrus in all treatment groups showed a percentage of 100% (Table 1). The pregnancy rate of ewes in each treatment group was 85% after natural mating, transcervical artificial insemination and endoscopic artificial insemination (Table 2). Evaluation of pregnancy checks in ewes using transrectal USG Dawei Vet 6, on day 30 showed twin embryos with a hyperecogenic period (Figure 2).

Table 1. The level of oestrus occurrence in dorper sheep

Treatment group	**Cement dosage (cc)**	**Average time of oestrus (hours)**	**Incidence of estrus on day 17 (%)**
P1	0.5	55	100
P2	0.25	55	100
P3	0.1	55	100

Table 2. Pregnancy rate in dorper sheep through ultrasound examination

Treatment group	**Total sheep**	**Number of pregnant sheep**	**Pregnancy percentage (%)**
P1	20	17	85
P2	20	17	85
P3	20	17	85

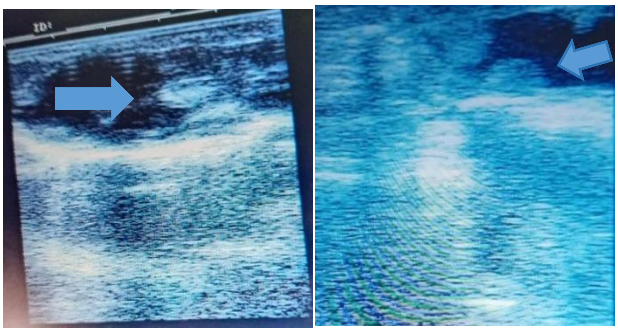

Figure 2. Pregnancy examination in dorper sheep with transrectal ultrasound dawei vet 6 on day 30. The arrow shows twin embryos with hyperecogenic nuances.

Discussion

A trial of administering PGF2α (dinoprost) was carried out to evaluate the efficacy of a prostaglandin-based estrus synchronization protocol in ewes so that they come into estrus more quickly [12]. Every 1 ml of dinoprost tromethamine is equivalent to 5 mg of dinoprost. A dose of 5 mg dinoprost is able to induce estrus in most dorper and crossbreed sheeps, resulting in the highest lambing percentage in Indonesia local sheeps so this dose is recommended for synchronizing estrus in dorper sheeps [13]. Dinoprost prostaglandin has a lutalyse effect with a lower dose of 5 mg capable of causing symptoms of lust within 55 – 96 hours after removing the sponge. Treatment with an intravaginal sponge using Medroxy Progesterone Acetate (MPA) for 12 – 14 days and administration of Equine chorionic gonadotrophin (eCG) 200 IU is carried out to increase the secretion of Luteinizing Hormone (FSH-LH), follicle development, and estradiol production which causes estrus to occur. an LH surge occurs and ovulation occurs more quickly [14].

Experiments on administering PG 600 were carried out to stimulate ovarian follicles to produce mature egg cells (ovum) and increase signs of estrus (heat) [15]. This causes the release of a mature egg from the ovarian follicle (ovulation), and promotes the formation of corpora lutea, which is necessary to maintain pregnancy once the animal becomes pregnant [16]. Progesterone sponge treatment with eCG for the onset of estrus in ewes was shown to show a good response based on the appearance of estrus. Progesterone sponge using MPA was given for 14 days and withdrawn on the 17th day and at that time a combination of PG 600 injection with a dose of 200 IU and 5 mg dynoprost intramuscularly showed excellent lust. Real-time transrectal ultrasound scanning of sheeps applied between days 24 and 34 of gestation offers a safe, accurate and practical way to determine the number of fetuses and stage of pregnancy thereby allowing more appropriate feeding and management in the later stages of pregnancy [17].

To determine the position of the uterus for injection with a 26 G needle made elongated through a special trocard for endoscopy. The reproductive tract of the sheep's cornua is usually located frontal to the bladder tract [18]. In sheep experiencing estrus, it can be characterized by hyperemia in the reproductive tract [3]. The ducts appear pale to dark pink and will curve when touched by endoscopic instruments, the location of the ducts and their relationship to surrounding structures need to be considered to facilitate safe endoscopic artificial insemination injections [19]. Another thing that needs to be considered before carrying out endoscopic artificial insemination is that the sheep must be fasted for 12 – 24 hours to empty the intestines so that they do not cover the uterus [4]. In addition, the sheep's stomach is filled with food, causing pressure on the uterine horn or uterine cornua due to enlargement of the rumen, making it difficult to inject dorper sheep semen [20].

Pregnancy in local Indonesian sheep has been detected in sheep before 30 days of gestation using dawei 6 Sono vet ultrasonography. Lower accuracy and false negative diagnoses on days 15-30 may be due to the intrapelvic location of the conceptus in the first month of pregnancy and the smaller size of the conceptus making it difficult to visualize via the trans-abdominal approach [21].

During early pregnancy, the ovine conceptus has a fibrous structure with a small amount of fluid that occupies the uterine horns and body of the uterus [22]. As the pregnancy progresses, the developing conceptus and placenta become visible ultrasonically which helps in diagnosing pregnancy more accurately [23]. The rapid growth of the conceptus after day 30 which makes it more visible ultrasonographically may be the reason for the significantly increased sensitivity and specificity of this technique [24]. Reasons for false positive diagnosis may be due to pyometra, hydrometra, and early embryonic death which is highest between days 3-26 of pregnancy [25].

Tool-assisted reproductive technology is used in animal reproduction to encourage efficient use of germplasm to increase the genetic value of companion and production animals [26]. Endoscopic artificial insemination is a reproductive technology that has revolutionized the dorper sheep farming industry [27]. The success of an endoscopic artificial insemination program depends on the implementation of an appropriate estrus synchronization program, selection of ewes, and thorough knowledge of reproductive physiology [3]. In addition, appropriate equipment and adequate surgical expertise help reduce morbidity and mortality rates in sheep [28]. Endoscopic artificial insemination can be associated with several complications due to inadequate ewe preparation, poor technique, or equipment failure [1]. Therefore, careful planning is essential to carry out procedures safely and with consistent production success rates.

The use of endoscopic artificial insemination techniques is more efficient than natural mating techniques and transcervical artificial insemination techniques for dorper sheep offspring produced in large populations. This can be explained because in natural mating using superior males it must be in a large population considering the limited ability of superior dorper rams to mate with local synchronized sheep in large numbers whereas in transcervical artificial insemination the ability of the inseminator is very limited considering the many differences in the cervical opening and anatomical size. cervix in each local sheep [4].

The addition of endoscopic artificial insemination to small ruminant farming practices can be very profitable, but requires large investments in the form of purchasing equipment and adequate training of veterinarians and field technical staff [3]. Performing endoscopic artificial insemination requires teamwork and good coordination between veterinarians trained to perform endoscopic artificial insemination, operators trained to handle semen processing, and other personnel taking part in handling and preparing the lamb for surgery [6]. It takes some effort to get the team used to the flow of the procedure. However, if the operator and team are skilled at carrying out the process, it is possible to inseminate as many as 100 to 200 sheep per day [29]. The addition of endoscopic artificial insemination to small ruminant farming practices may be an economically viable option and can yield immediate benefits, as producers see increased pregnancy rates even with the use of small doses of semen.

Endoscopic artificial insemination procedures in small ruminants may be performed provided that the surgical standards performed comply with animal welfare requirements [27].

Endoscopic artificial insemination should be carried out by a registered veterinarian because it requires trocarization skills to insert semen into the uterus of the local sheep [1]. Where state or territory legislation permits endoscopic artificial insemination to be carried out by lay operators then the supply of veterinary medicines will need to be restricted to lay operators for use in accordance with relevant state or territory legislation [3]. Some of the conditions for sheep that can be subjected to endoscopic artificial insemination are that local sheep are healthy and not pregnant, and proven to have no genetic defects [30]. Operators or veterinarians using endoscopic artificial insemination techniques have a responsibility to maintain and regularly update knowledge regarding advances in the field.

Conclusion

In the future, the use of endoscopy will require adequate veterinary skills. The use of endoscopic artificial insemination techniques is more efficient than natural mating techniques and transcervical artificial insemination techniques for Indonesian local sheep offspring produced in large populations with using semen Dorper. It seems that natural mating using superior males must be in a large population considering the ability of superior rams to mate with large numbers of synchronized sheep, whereas transcervical artificial insemination is very limited in that the inseminator's ability to see the target hole in the area around the linea alba in addition to the different anatomical sizes of the cervix.

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