

# Laparoscopic artificial insemination in sheep: review and cost benefit analysis

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## Abstract

Artificial insemination in sheep has vastly increased in popularity as preserved semen is readily available. Laparoscopic artificial insemination (LAI), a surgical procedure, involves deposition of semen directly into the uterus using a laparoscope. We have reviewed LAI, compared benefits to natural breeding and transcervical artificial insemination, described procurement, and provided a cost benefit analysis. LAI is an advanced assisted reproductive technique with several benefits, including higher pregnancy rates requiring fewer sperm per breeding than vaginal or transcervical artificial inseminations. Although LAI involves a higher start-up cost compared to other techniques, it is an economically feasible option. With the continual development of new equipment, techniques, and semen preservation technologies, LAI can enhance herd genetics without investment in expensive genetically superior studs and maintaining them. LAI with an appropriate estrus synchronization protocol results in higher numbers of superior quality lambs per ewe for higher profit margin and higher lambing rates for producers, making LAI a profitable service for a veterinary practice.

**Keywords:** Sheep, artificial insemination, laparoscopy, fertility, economic analysis

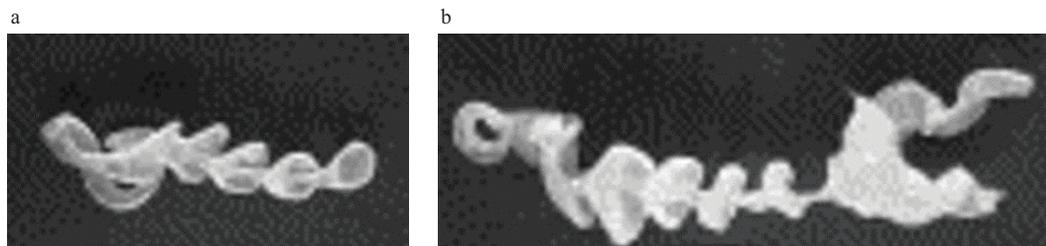
## Introduction

Artificial insemination (AI) technologies have dramatically increased the ability of producers to shift the genetics of their herd to meet milk and meat market demands.<sup>1</sup> Development of AI and semen preservation has allowed producers to access top-tier genetics from around the world.<sup>2</sup> In the small ruminant industry, AI is a high-impact assisted reproductive technology. It supports genetic improvement and contributes to conservation of genetic resources. In addition, it is a safe and effective method of reproduction while contributing to animal health by controlling the spread of several infectious diseases.

The successful implementation of AI depends on effective animal management, semen evaluation and preservation, estrous cycle control, estrus detection, and appropriate insemination methods. Although the use of AI dates to the 1700's, it was not heavily researched or used until the 1900's.<sup>3</sup> Now, AI is a widespread practice and is used throughout the world. Many variations and methods of AI have been developed, with species responding differently to each type of approach.

Laparoscopy artificial insemination (LAI) was first reported in the 1980's.<sup>4,5</sup> In ewes, unlike cows, the major limiting factor is

the difficulty to pass the insemination pipette through the cervix for intrauterine semen deposition. Ewe's cervix is small, narrow, rigid, and tortuous (Figure 1)<sup>6</sup> and it is relatively more difficult to navigate the inseminating pipette without transrectal cervical manipulation. For this reason, semen is usually deposited at cervical entrance or in the cervix (intracervical) resulting in poor pregnancy rates. Not only cervical navigation is bypassed in LAI, it also results in higher pregnancy rates compared to vaginal artificial insemination (VAI) and transcervical artificial insemination (TCAI).<sup>7,8</sup> For this reason, fixed time intrauterine insemination is performed via laparoscopy; commonly used in large-scale breeding programs worldwide. Although LAI is a minimally invasive procedure it requires veterinary expertise and raises animal welfare concerns and demands specialized equipment and more labor compared to cervical insemination. Although several alternative approaches have been proposed to replace laparoscopy, LAI technique continues to be the gold standard when higher pregnancy rates are required. In small ruminants, there are 3 types of AI techniques: vaginal (pericervical), cervical (intracervical) or intrauterine (transcervical and laparoscopic technique) semen deposition.<sup>7</sup> Objectives were to: 1. review LAI literature; 2. compare benefits of laparoscopic artificial insemination over natural breeding and TCAI; 3. describe the procedure; and 3. perform economic analyses and determine the break-even point for LAI service to become profitable.



**Figure 1.** Image of mold of ewe's cervical canal with cervical folds and rings (Courtesy of Dr. Brian Buckrell)

### Intravaginal (pericervical) artificial insemination

Vaginal insemination is performed with fresh or chilled semen. In this method, the semen is deposited in the cranial vagina (fundus) using an infusion rod. The procedure is easy and quick to perform under field conditions and may result in lower but still acceptable pregnancy rates. Vaginal AI is best suited to use after estrus detection during the natural breeding season. The ideal timing of AI is before ovulation (i.e. 12-18 hours after estrus onset);<sup>9</sup> recommended semen volume is 0.2-0.5 ml and the number of progressively motile sperm is minimum of  $300 \times 10^6$ .<sup>7,10</sup>

Intravaginal AI is effective in ewes with fresh semen compared to extended (chilled) semen. However, conception rates after vaginal insemination are lower with pharmacological estrus synchronization. Additionally, pregnancy rates following vaginal insemination with frozen semen are not acceptable. Cervical insemination with fresh or liquid semen and intrauterine insemination with frozen semen via laparoscopy require smaller insemination doses.

### Intracervical artificial insemination

Intracervical AI with fresh or chilled semen is the ideal choice in small ruminants since the external cervical os is located/visualized with illumination. The insemination pipette is passed through the speculum into the cervix, without excessive force, to a depth of 5-12 mm. Semen is deposited after positioning the pipette in the cervix. Special attention is necessary to minimize semen backflow. Unlike intravaginal AI, conception rates achieved with fresh or chilled semen via this method are acceptable after pharmacological estrus synchronization.<sup>7,9,11</sup> The ideal time for AI is 48-65 hours after intravaginal progesterone inserts removal or 15-17 hours after the onset of detected estrus.<sup>12-14</sup> Originally, use of frozen semen was limited because of the low lambing percentages (25-35%) with cervical AI. This was associated with the reduced viability of frozen sperm resulting in lower numbers of viable or undamaged sperm reaching fertilization site. Currently, with the development of cervical AI skills and good semen evaluation and processing methods, pregnancy rates are improved.<sup>15</sup> Insemination volume and the number of motile sperm required are 0.2-0.5 ml and  $150-200 \times 10^6$ , respectively.<sup>7,10</sup> Since LAI is prohibited in most of the North European countries and requiring special and costly equipment and well-qualified technicians, importance is given to cervical AI with frozen semen.<sup>16,17</sup> In goats, intracervical AI is the most widely practiced method since caprine cervix is relatively easier to traverse than ovine cervix.

### Intrauterine artificial insemination

Lack of perfect nonsurgical procedures for ewes severely limits its use. Direct transfer of semen into uterine lumen with the

laparoscopic technique has resulted in acceptable results, but it has disadvantages. Alternatively, transcervical intrauterine AI techniques (e.g. Guelph system of transcervical AI) have been developed.<sup>10,18-24</sup> Transcervical AI procedure require special positioning of the animal, cervical retraction and stabilization, and specially designed instruments for stabilization and passing insemination pipette through the cervix. Recently developed methods allow the semen to be deposited atraumatically, even deeper into the uterine horns by the transcervical route.<sup>20-22</sup> However, the process of manipulating an AI catheter through the cervix still has been linked to reductions in pregnancy and lambing rates.<sup>22</sup> It has been hypothesized that cervical trauma and vaginal/cervical stimulation caused by the catheterization may activate pathways interrupting pregnancy between days 3-14.<sup>21,22</sup> Intracervical application of hyaluronan 52 hours after sponge removal improved cervical relaxation and increased cervical entry.<sup>25</sup> Among other methods, the use of drugs (hormones,<sup>26,27</sup> chemokines,<sup>28</sup> myorelaxants,<sup>29</sup> beta-adrenergic blocking agents<sup>30,31</sup>) and the design of inseminating catheters<sup>32,33</sup> associated with manipulation of the cervical canal (i.e. Guelph System<sup>17-20</sup>) have been investigated with some success. The procedure of transcervical AI could be even simpler if it were sufficient to deposit the semen in only 1 uterine horn. There are opinions to the contrary,<sup>5</sup> but evidence from LAI in sheep indicates that the deposition of semen in a single uterine horn leads to fertilization of ova from both ovaries.<sup>34</sup> Insemination volume and the number of motile sperm required are 0.2-0.5 ml and minimum  $100-200 \times 10^6$ , respectively.<sup>7,10</sup> Trained and experienced inseminators may enter the cervix in 75-85% of ewes.<sup>20,21</sup> However, transcervical AI cannot be used with an acceptable success rate in nulliparous ewes. Cervical injury, abscesses, infections and poor pregnancy rates are associated with this technique, but they vary by operator, semen dose, ewe condition, and experience.<sup>22,24</sup> Pregnancy rates are generally lower than those achieved by laparoscopic AI (fresh: 40-70%; frozen: 30-70%).

### Laparoscopic intrauterine AI

The novelty of laparoscopic intrauterine insemination of ewes represents the most remarkable development in ovine AI industry. With the laparoscopic technique, decreased amount of semen is deposited into uterine horns lumen. The number of sperm required for each insemination is lower and the volume of semen is proportionally higher.<sup>35,36</sup> This allows more suitable dilution rates and, therefore, better preservation and protection of sperm. Laparoscope-aided intrauterine AI improves pregnancy rate achieved with cryopreserved semen and facilitates the widespread dissemination of valuable genotypes. When correctly performed, this method has a success rate of > 60%, but this rate will vary depending on the quality of semen, the time of the year, the ewe's condition, and the skill of the inseminators. Conception rates achieved with frozen semen used by this method are

**Table 1.** Factors influencing the reproductive outcome

Factors	References
Female factors:	
Age of the ewe/parity	39-41
Genetics	42
Ewes' body condition	43,44
Prolificacy	45,46
Health management	47
Nutritional management	48-51
Synchronization and induction of estrus	52-54
Stress	55,56
Hygiene	57,58
Animal management	59,60
Male factors:	
Age of the ram	61
Genetics	42
Scrotal circumference	62,63
Superior quality semen	6,41,61,64,65
Others:	
Farm	41,66
Year	41
Season	41,66,67
Semen type	68,69
AI technique	66,68
AI technician	41,66
Insemination time, method	6,67,70
Interval from lambing to AI	41

higher than for intracervical insemination, because of better cryopreservation of sperm and deposition of semen in the uterus. The main disadvantages are the need for expensive laparoscopic equipment, invasive surgery (animal welfare issues), and the technical expertise needed to perform the procedure. Its cost usually limits this procedure for expensive semen, valuable animals or breeding companies. Current techniques recommend depositing half the total dose of semen in the middle region of each uterine horn without regard to ovulation site;<sup>9,37</sup> however, deposition in 1 horn had acceptable conception rates.<sup>8,37</sup>

In estrus-synchronized ewes, ideal timing of AI is 48-65 hours<sup>12-14,37</sup> after the removal of progesterone inserts and 12-18.5 hours<sup>38</sup> after estrus detection. Insemination volume and the number of motile sperm required are 0.25 or 0.5 ml and minimum of 20-40 × 10<sup>6</sup>, respectively.<sup>9,15,16,38</sup> An average ejaculate diluted appropriately can be utilized to inseminate 50 ewes. An experienced team in a well-equipped and organized operation can inseminate 250-500 ewes in a day with this technique.

### Factors influencing the reproductive outcome

Application of AI in sheep production requires appropriate knowledge of animal management, seasonality, breeds involved, and production system of small ruminants. Several factors influence AI program success in small ruminants (Table 1).

**Table 2.** Length of breeding season and breed of sheep

Length of breeding season	Breeds
Long (6-8 months)	Dorset Finn Polypay Rambouillet Rideau Romanov
Medium (4-6 months)	Canadian Charollais Hampshire Oxford Suffolk
Short (4 months)	Leicester North Country Cheviot Scottish Blackface Shetland Texel

Sheep breed can influence the length of breeding season; sheep breed should be selected based on operation objective. In accelerated breeding programs (increase the number of lamb crops per ewe; 3 lambing in 2 years and 5 lambing in 3 years) it is ideal to have sheep breeds with a long breeding season. Sheep breeds and breeding season length are provided (Table 2).<sup>71,72</sup>

## Laparoscopic AI

### Animal selection

Careful selection of animals is vital to achieve good reproductive outcome and to deliver the best monetary results to clients. As mentioned above, there are several factors pertinent to ewe and ram, among others that influence LAI results.

Selection of ram and ewe provides the best improvement in genetics and conception rate. Implementing AI allow producers with an opportunity to choose genetically superior semen with desirable traits, based on operation objectives. Frozen semen technology provides the ability to obtain semen from genetically top studs throughout the world. However, reproductive performances with fresh, cooled, and frozen semen can differ (Table 3).

### Guidelines for animal welfare

Animal welfare is important in all aspects of animal husbandry and veterinary medicine. Animal health and well-being should be considered in breeding protocol and AI. Precautions should be taken to prevent undue stress and pain. Sheep should be sedated for LAI and given preemptive pain medication and prophylactic antibiotics.<sup>78</sup> Effort should be made to maintain sterility of all instruments entering the abdominal cavity.<sup>78</sup>

**Table 3.** Reproductive outcome in laparoscopic intrauterine insemination\*

Semen type	Insemination dose (x 10 <sup>6</sup> sperm)	Number of ewes	Reproductive outcome (%)	Season	References
Fresh	50 in each horn	300	PR 70.1	Breeding	73
	N/A	2,508	PR 82.2	Breeding and anestrus	69
Cooled	20 in each horn	265	LR 75.3	Breeding and out of season	74
Frozen	50	103	LR 62.1	Out of season	75
	75	243	PR 50.2	Breeding	76
	N/A	25,939	PR 70.1	Breeding and anestrus	69
	80	198	LR 63.6	Breeding	77

\*synchronization regimens and insemination timing referred were different; PR: pregnancy rate, LR: lambing rate

The animal should be in the cradle only for a short duration to prevent adverse effects associated with dorsal recumbency. Time spent in the Trendelenburg position should be minimal due to pressure on lungs. Therefore, animals should only be positioned when everything is ready. If an animal develops breathing complications, it should be returned to a normal position. After LAI, sheep should be monitored for postanesthetic complications. When LAI is performed correctly, complications are mitigated with lower incidence of surgical complications. A comprehensive list of LAI complications is provided.<sup>37</sup> Complications include rupture or puncture of abdominal viscera, subcutaneous emphysema, hematoma or subcutaneous bleeding, abscess formation, peritonitis, sepsis, intra-abdominal adhesions, and uterine bleeding.<sup>37</sup>

### Synchronization of estrus

Synchronization of estrus is vital for AI program success. Ewes are often artificially inseminated at a fixed time. The basis for implementation of synchronization protocols is to hormonally regulate the estrous cycle at the herd level; this facilitates scheduling of the procedure at a fixed time and increases fertility. Various synchronization protocols are available for small ruminants; protocol used should be customized with the owner's and veterinarian's goals and schedules.

There are several pharmaceuticals available in the USA for synchronization protocols. Prostaglandin (PGF<sub>2α</sub>) lyses a corpus luteum in cycling ewes to induce estrus and is given twice (7-12 days apart) for luteal regression and estrus expression (Table 4).<sup>79-82</sup> Estrus detection is required for the 2-dose PGF<sub>2α</sub> protocol. Since estrus expression occurs over a period, timed AI is not possible. It was not successful due to poor fertility and embryonic loss<sup>83</sup> compared to synchronization protocols with progesterone, progesterone, and eCG,<sup>84</sup> however, there was no impact on fertilization.<sup>85</sup> Efficiency of PGF<sub>2α</sub> in combination with the ram effect was explored.<sup>86</sup>

The most common method of estrus synchronization involves progesterone followed by a single dose of equine chorionic gonadotropin (eCG) or PG600. This requires progesterone, controlled internal drug release (CIDR)<sup>73</sup> or compounds with progesterone-like activity (progesterone sponges, MAP, medroxyprogesterone acetate,<sup>87,88</sup> or FGA, Fluorogestone acetate<sup>73,89</sup>) in intravaginal devices that are used 12-14 days prior to AI. At removal, a single injection of eCG<sup>73,90</sup> or PG600<sup>91,92</sup> is given subcutaneously to induce ovulation. Androgenized teasers can also be introduced to ewes along with eCG treatment to help stimulate estrus.<sup>93,94</sup> Most ewes will enter estrus 24-36 hours later, peaking at 48 hours.<sup>95</sup> Laparoscopic intrauterine AI of ewes with semen is normally performed

**Table 4.** Effect of prostaglandin F<sub>2α</sub> on estrus response and pregnancy in ewes

Prostaglandin F <sub>2α</sub>	Dose	Interval (days)	Season	Type of breeding	ER (%)	PR (%)	Reference
Cloprostenol	120 µg	7	Breeding	Natural	91	73.3	80
		9			94	75.9	
		11.5			94	75.9	
Cloprostenol	126 µg	9	Breeding		100		81
	68.25 µg	9			75		
	38.5 µg	9			87.5		
Dinoprost tromethamine	10 mg	Single dose	Breeding	Natural		60	82
Cloprostenol sodium	150 µg					70	
D-cloprosternol	150 µg					70	

ER: estrus response rate; PR: pregnancy rate

48-65 hours after CIDR removal (depending on whether fresh or frozen-thawed semen is used), with a minimum of  $25 \times 10^6$  motile sperm deposited per ewe.<sup>9</sup>

Several types of intravaginal devices resulted in varying reproductive outcomes. CIDRs are more accessible, quick to insert, and a more efficient form of progesterone.<sup>96</sup> However, protocols must be adapted depending on the progesterone and treatment form. CIDRs resulted in an earlier response and ovulation compared to sponges, 55 and 60 hours, respectively.<sup>95</sup> The delay in the commencement of ovulation was observed in November and December when most ewes are expected to be in anestrus.<sup>95</sup> CIDR treatment produced less variation around ovulation timing<sup>97</sup> and a greater tendency for higher conception rates, 69 compared to 58.6% with other intravaginal devices. Further, during breeding season, CIDR had significantly higher pregnancy, fertility, twinning rate and fecundity compared to sponges during breeding and out of season.<sup>73,98</sup> Another study, conducted during the breeding season, had higher fertility yields in short-term (6-7 days) CIDR-based treatments (75%) and the long-term (12-14 days) CIDR-based treatment (70%) than in the long-term (12-14 days) sponge-based treatment (45%).<sup>99</sup> Poor fertility in sponge groups was attributed to change in vaginal pH ( $\sim 8$ ) and vaginal microbiome (a higher incidence of *Salmonella* spp. and *Staphylococcus aureus*).<sup>99</sup> Conversely, the percentage of ewes lambing and twinning rate are similar between sponges and CIDR-treated ewes during breeding and out of season.<sup>96,100</sup> FGA sponges resulted in higher pregnancy than CIDRs in autumn (54.7 versus 67.4%), but not spring (59.2 versus 56.2%), suggesting a seasonal impact on the effectiveness of estrus synchronization.<sup>101</sup> However, another study reported no difference between pregnancy rates in March, April, and May inseminations compared to summer months.<sup>69</sup> The type of progestogen could also be a reason for the variability. A lower conception rate was observed with MAP (64.6%) compared to FGA (74.7%) or CIDR (71.7%).<sup>69</sup>

During breeding season, eCG treatment in ewes after progesterone removal increased rates of ovulation,<sup>102,103</sup> conception and lambing.<sup>73,103</sup> Treatment with 250 IU (72.9%) or 300 IU (79.1%) eCG doses resulted in higher pregnancy rate compared to 200 IU (62.4%). In nonseasonal ewes, a higher eCG dose (400 IU) at CIDR removal normally led to a superovulatory effect or increase in prolificacy, measured as twins and triplets.<sup>101</sup> During breeding season, GnRH treatment at estrus onset or before luteinizing hormone (LH) surge or 36 hours after progesterone device removal,<sup>104,105</sup> had no improvement in the number of pregnant ewes compared to current protocols<sup>97</sup> despite improved synchronization of anestrus ewes.<sup>102</sup> GnRH has been used in sexed semen<sup>106,107</sup> or in multiple-ovulation embryo transfer programs,<sup>102,108</sup> where timing of ovulation is critical for success. Estrus response and pregnancy for various combinations of progesterone and gonadotropin are provided (Table 5)

CIDR is the only approved form of progesterone device in the USA. Currently, eCG is either unavailable or backordered in the USA and PG600 does not perform as well as eCG. Standard doses of PG600, typically intended for pigs, can lead to overstimulation of the ovaries, resulting in reduced pregnancy rates due to issues with ovulation and embryo survival; therefore, a lower dose of PG600 is usually recommended for sheep.<sup>91,109</sup> Additionally, PG600 in small ruminants is considered extra-label. Therefore, other products, such as GnRH agonists (GnRH, hCG<sup>94</sup>) or FSH,<sup>110,111</sup> should also be considered. Combinations of various progesterone and gonadotropin

formulations to induce estrus for natural or artificial insemination in ewes are provided (Table 5).

### Postthaw semen evaluation

Evaluation of postthaw semen is important to determine the quality. Although postthaw motility is the primary criterion, evaluation of membrane integrity, viability and functional tests such as hypo-osmotic swelling and sperm DNA and acrosomal integrity are used to objectively evaluate sperm quality. However, in the field, motility and concentration are commonly used indicators to determine semen quality and quantity. Briefly, semen straws are removed from the liquid nitrogen storage tank and placed in a 37°C water bath for 1 minute, then dried with a paper towel. The straws are cut with scissors, and the samples are emptied into glass vials, which are placed on a 37°C digital dry block warmer and swirled for 1 minute. A 7.5  $\mu$ l sample is then pipetted to the iSperm (Madison, WI, USA) base chip for analysis of total motility and concentration.<sup>117</sup>

### Procedure

Laparoscopy requires insertion of a cannula/trocar through the abdominal wall, distension of the abdominal cavity with sterile air or CO<sub>2</sub>, and visual examination of the abdominal organs with an illuminated telescope. In LAI, 2 ports of entry are created in the abdomen: 1 for the laparoscope to visualize the uterus and the other for passing the AI gun for insemination. Equipment list for transcervical artificial insemination and laparoscopic insemination in small ruminants is provided.<sup>118</sup> The procedure is relatively quick once ewes are ready for AI. The procedure can take < 1 minute to a few minutes per animal, depending on experience. It is advisable to have several working groups: technicians to help sedate ewes, load them into the cradle and prepare ewes for LAI and monitor them; technician to thaw and evaluate semen; personnel to perform AI; and a group to monitor recovery.

Sedation can be accomplished through a single intravenous injection of an anesthetic combination. A mixture of 1,000 mg of ketamine, 100 mg of xylazine ( $\alpha$ -2 adrenoceptor agonist, Table 6), and 10 mg of butorphanol. A dose of 0.1 ml/20 lbs can sedate a small ruminant long enough to undergo the LAI procedure while maintaining minimal side effects and short recovery times.<sup>119</sup>

Intramuscular (0.05-0.3) or intravenous (0.016-0.1) xylazine alone or in combination with butorphanol (intravenous xylazine [0.1-0.2] + intravenous butorphanol [0.01-0.02]) or (intramuscular xylazine [0.02] + intramuscular butorphanol [0.05-0.07]) are used.<sup>121,122</sup> Xylazine and other  $\alpha$ -2 agonists have several dose-dependent adverse effects and require careful consideration.<sup>123</sup> For instance, small ruminants are particularly sensitive to the pulmonary effects of these drugs. The primary cause of these pulmonary effects is the activation of pulmonary intravascular macrophages (PIMs). Once activated, PIMs release prostaglandins and other vasoactive substances leading to alveolar edema, increased transpulmonary pressure, reduced pulmonary compliance, and pulmonary congestion.

Ketamine (1 mg/kg) combined with xylazine (0.05 mg/kg; 20 mg/ml) and butorphanol (0.025 mg/kg) are used to make a stock solution for the LAI procedure.<sup>120</sup> Ewes are given intramuscularly 0.02 ml/kg. After 10 minutes, the sedated females are ready to stand alongside of an artificial insemination table and for rolling into dorsal recumbency.

**Table 5.** Effects of combinations of various progesterone and gonadotropin formulations on estrus response and pregnancy following natural or artificial insemination in ewes

Progesterone	P <sub>4</sub> treatment duration (days)	Gonadotropins prostaglandins	Season	Breeding type	ER (%)	PR (%)	Reference
FGA 40 mg	14	eCG 200 IU at P <sub>4</sub> withdrawal	Breeding season	Natural 1:5 - ram:ewe	93.3	60.0	89
FGA 40 mg	5	eCG 200 IU at P <sub>4</sub> withdrawal + cloprostenol 100 mg at P <sub>4</sub> withdrawal			86.7	86.7	
FGA 40 mg	5	Cloprostenol 100 mg on day 0 + eCG 200 IU at P <sub>4</sub> withdrawal	Breeding season	Natural 1:5 - ram:ewe	92.3	92.3	
FGA 40 mg	5	Cloprostenol 100 mg on day 0 + GnRH 100 mg 30 hours after P <sub>4</sub> withdrawal			66.6	33.3	
-	-	GnRH 100 mg on day 0 + cloprostenol 100 mg on day 5 + 200 IU on day 5			91.7	66.7	
FGA 40 mg	5	Cloprostenol 100 mg on day 5 at P <sub>4</sub> withdrawal	Breeding season	TAI (+ 52 hours or + 60 hours after P <sub>4</sub> withdrawal)		52 hours: 50.0 60 hours: 33.3	89
FGA 40 mg	5	Cloprostenol 100 mg on day 5 at P <sub>4</sub> withdrawal + GnRH (100 mg given 30 hours after P <sub>4</sub> withdrawal)				52 hours: 40.0 60 hours: 20.0	
FGA 40 mg	5	Cloprostenol 100 mg on day 5 + eCG 200 IU on day 5 at P <sub>4</sub> withdrawal + GnRH (100 mg given 30 hours after P <sub>4</sub> withdrawal)				52 hours: 20.0 60 hours: 60.0	
CIDR 0.3 g	14	eCG 600 IU at P <sub>4</sub> withdrawal	Breeding season	TLAI	96.9	77.8	73
FGA 30 mg	14	eCG 600 IU at P <sub>4</sub> withdrawal	Out of breeding season	Natural 1:6 - ram:ewe	95.7	62.4	88
MAP 60 mg	12	Control			-	56	
MAP 60 mg	12	eCG 500 IU at P <sub>4</sub> withdrawal	Out of breeding season	Transcervical AI	-	88	90
CIDR 0.3 g	14	eCG 400 IU at P <sub>4</sub> withdrawal			100.0	75.0	
		GnRH 400 mg at P <sub>4</sub> withdrawal	Breeding season	Natural mating 3:16 - ram:ewe	100.0	75.0	91
		GnRH 500 mg at P <sub>4</sub> withdrawal			100.0	70.0	
		GnRH 600 mg at P <sub>4</sub> withdrawal			100.0	75.0	
		hCG 200 IU at P <sub>4</sub> withdrawal			100.0	75.0	
		hCG 300 IU at P <sub>4</sub> withdrawal			100.0	80.0	
		hCG 400 IU at P <sub>4</sub> withdrawal	100.0	75.0			
CIDR 0.3 g	9	Cloprostenol 120 mg on day 7 + PG600 5 ml	Breeding season	Natural mating 3:16 - ram:ewe	-	77.0	91
		Cloprostenol 120 mg on day 7 + PG600 1.5 ml			-	87.5	
		Cloprostenol 120 mg on day 7 only			-	37.5	

(Continued)

Table 5. (Continued)

Progesterone	P <sub>4</sub> treatment duration (days)	Gonadotropins progestandins	Season	Breeding type	ER (%)	PR (%)	Reference
CIDR 0.3g	5	3 ml PG600 (240 IU eCG, 120 IU hCG) at P <sub>4</sub> withdrawal	Anestrus	Natural mating 1:25 – ram:ewe	79.2	71.9	92
		Control				66.7	
CIDR 0.3g	5	3 ml PG600 (240 IU eCG, 120 IU hCG) 1 day before P <sub>4</sub> withdrawal	Breeding	Cervical AI	79.6	51.7	54
		Control				84.3	
FGA 45 mg	11	0.1 mg of PGF <sub>2α</sub> on day + 6 µg of intramuscular GnRH 36 hours after sponge withdrawal	Breeding	Cervical AI	70.0	71.4	54
	11	330 IU and 0.1 mg of PGF <sub>2α</sub> day 9				83.3	
	11	330 IU and 0.1 mg of PGF <sub>2α</sub> on day 9 + 6 µg of intramuscular GnRH 36 hour after sponge withdrawal	Breeding	Natural	86.7	80.8	112
	13	0.1 mg of PGF <sub>2α</sub> on day 12 + 300 IU at sponge withdrawal				80.0	
	13	300 IU at sponge withdrawal	Nonbreeding	Natural	76.7	73.91	112
MAP 60 mg	14	eCG 300 IU at P <sub>4</sub> withdrawal				80	
MAP 60 mg	14	eCG 600 IU at P <sub>4</sub> withdrawal	Nonbreeding	Natural	80	100	112
Control	-	-				10	
MAP 60 mg	6	eCG 300 IU + 75 µg D-cloprostenol, 1 day before P <sub>4</sub> withdrawal	Breeding	Natural	72.7	45.5	113
	9	eCG 300 IU + 75 µg D-cloprostenol, 1 day before P <sub>4</sub> withdrawal				72.7	
	12	eCG 300 IU + 75 µg D-cloprostenol, 1 day before P <sub>4</sub> withdrawal	Nonbreeding	Natural	80	20	114
FGA 40 mg	12	eCG 140 IU 1 day before P <sub>4</sub> withdrawal				100	
FGA 40 mg	12	eCG 280 IU 1 day before P <sub>4</sub> withdrawal	Breeding	Natural	100	73.7	103
FGA 40 mg	10	CON				89	
FGA 40 mg	10	eCG 100 IU	Breeding	Natural	92.7	58.8 <sup>a</sup>	103
FGA 40 mg	10	eCG 200 IU				95.8	
FGA 40 mg	10	eCG 400 IU	Breeding	AI	91.7	100 <sup>b</sup>	115
FGA 40 mg	14	eCG 500 IU				100	
FGA 40 mg	14	eCG 600 IU	Breeding	Natural	100	93.7 <sup>b</sup>	116
FGA 40 mg	14	eCG 750 IU				100	
Control	-	-	Breeding	Natural	97.1	79.4 <sup>c</sup>	116
FGA 40 mg	12	eCG 300 IU				64	
		eCG 450 IU	Breeding	Natural	77.7	19.3	116
		eCG 600 IU				86.3	
		eCG 750 IU	Breeding	Natural	80.9	19	116

CIDR: controlled internal drug release; FGA: fluorogestone acetate; MAP: medroxyprogesterone acetate; ER%: estrus response rate; PR%: pregnancy rate; AI: artificial insemination; TAI: timed artificial insemination; TLAI: timed laparoscopic artificial insemination; treatments without common superscripts differed ( $p < 0.05$ ).

**Table 6.**  $\alpha$ -2 adrenoceptor agonist and doses<sup>120</sup>

Drug	Treatment route	Dose
Dexmedetomidine ( $\mu\text{g}/\text{kg}$ )	intravenous	5-10
	intramuscular	10-30
Detomidine ( $\mu\text{g}/\text{kg}$ )	intravenous	3-20
	intramuscular	20-30
Romifidine ( $\mu\text{g}/\text{kg}$ )	intravenous	3-40
	intramuscular	40-80
Xylazine	intravenous	0.016-0.1
	intramuscular	0.05-0.3

Intramuscular (0.05-0.2 mg/kg) or intravenous (0.02-0.05 mg/kg) acepromazine is the most used agent in this group. Compared to xylazine, it has a delayed onset of action. The maximum effect after intravenous treatment may take 15-20 minutes, and its duration of action lasts 4-6 hours. Although acepromazine has minimal respiratory effects, it may cause arterial hypotension, especially at higher doses or in hypovolemic animals. Furthermore, acepromazine lacks analgesic properties, and its sedative effect is weaker than xylazine. Acepromazine is most effective when used in combination with other drugs. Benzodiazepines (intravenous diazepam: 0.5-1.0 mg/kg given slowly, and intravenous, subcutaneous, or intramuscular midazolam 0.3-0.5 mg/kg) have minimal effects on the cardiovascular and respiratory systems;<sup>124</sup> used for sedation in small ruminants as the sole drug, but they are preferred in combination with other agents.<sup>125</sup>

Once ewes are sedated and loaded onto the cradle on dorsal recumbency, the posterior ventral abdomen (30 × 30 cm area cranial to the udder) is surgically prepared (clipped and scrubbed with chlorhexidine- and sterile saline-soaked gauze).

Once the site is sterilized, injection of local anesthetic (2% lidocaine) is given 5 inches cranial to the teats and 2 inches off the midline on each side with care to avoid large veins. After a few minutes (for the local anesthetic to take effect), the cradle is lifted to a 40 degree angle, with the abdomen at a higher elevation than the head (Figure 2). A small incision through the skin (but not the abdominal wall) is made in each of the spots where the local anesthetic was injected. A trocar is carefully passed through the abdominal wall along with cannula and removed to insufflate the abdomen with CO<sub>2</sub> before passing a second trocar through the second incision. The trocar is removed from the second cannula and a laparoscope is passed through 1 canula while forceps is passed through the other (Figure 2). Once the uterus is visualized through the laparoscope, it is manipulated into position for insemination with forceps. Once the uterus is secured in a desirable position, the forceps is withdrawn and replaced with the insemination gun loaded with semen. The insemination gun is used to puncture and deposit semen in both uterine horns at the midway point between the utero-tubal junction and the base of the horn. Once this is accomplished, equipment is withdrawn from the abdomen, abdomen deflated, and incisions are closed with staples, sutures, or tissue glue; however, sutures and staples are avoided.<sup>37</sup> Ewes should be allowed to recover in a quiet pen while monitored for adverse effects. In general, ewes readily walk away. Intravenous yohimbine (0.1-1.0 mg/kg) is the antidote for xylazine to reverse effects. Intramuscular or intravenous atipamezole may also be used for reversal (0.1-0.2 mg/kg). Diazepam and midazolam are reversed with intramuscular or intravenous flumazenil (0.02 mg/kg).<sup>126</sup>

#### Estimated input cost for laparoscopic artificial insemination service

Purchasing the reusable equipment to perform LAI is an investment upfront but can quickly turn into profit. To perform LAI, it is necessary to have the equipment to enter the abdomen, visualize the uterus and deposit the semen in the



**Figure 2.** Laparoscopic artificial insemination procedure in an ewe (Courtesy of Dr. Salman Waqas)

lumen of the uterus. Although 5.0, 6.5, 7.0, 7.5, and 10 mm laparoscopes are available, 5 mm is a popular choice among veterinarians and will be the size of equipment mentioned unless otherwise mentioned. Purchasing refurbished or used medical equipment has been a popular choice and its price is used in our cost analysis. Necessary reusable equipment includes, laparoscope, trocars, cannulas, a CO<sub>2</sub> tank, scope, light source, forceps, a laparoscopy cradle, LAI gun, and semen processing equipment.

Laparoscope with light source and forceps: cost of a laparoscope can vary depending on whether it provides digital imaging or a lens, degree of offset, and type of light source; new \$7,000 or used for \$2,500. A 0-degree scope with a lens is ~ \$250. A used halogen light source with a fiber optic cable is ~ \$1,000 or a portable handheld endoscope light source (~ \$150) may be more suitable for field use. Laparoscopic forceps is required to manipulate the uterus or omentum before insemination and can be purchased for ~ \$100.

Trocar and cannulas: available in 3 sizes: 5, 7.5, and 10 mm. It is desirable to have smaller size cannulas as it allows for smaller incisions and smaller rigid 5 mm laparoscopes, require no trocar adaptors for AI guns, carry reduced risks of abdominal perforation and prolonged lifespan due to lesser wear and tear. Trocars and cannulas with a port are required to enter and insufflate the abdomen; 2 are needed as 1 will be for the scope and the other for the forceps to manipulate the uterus or insemination pipette. A set of these can be ~ \$500 for a new set or \$250 for a used set.

CO<sub>2</sub> tank: is required to insufflate the abdomen. Insufflating the abdomen allows for more space to manipulate and visualize the uterus. A medical grade insufflation air unit or a generic tank and regulator is used. Medical grade unit, CO<sub>2</sub> tank with regulator, and a commercial vacuum pump can be purchased for \$8,750. A used tank and regulator with a hose can be purchased for ~ \$300.

Cradle: is needed to hold the sheep in dorsal recumbency at an incline during the procedure. A new cradle can be purchased for ~ \$1,800. Used units are not always readily available but can sometimes be purchased for < \$1,000. Although 2 cradles are ideal, only 1 cradle was considered for economic analysis.

An LAI gun for use with Robertson insemination pipette, sheaths and semen straws (Minitube USA, Inc, Verona, WI, USA) can be purchased as a new set for \$400 or conversely a less costly Robertson insemination pipette with a syringe can be used for semen deposition. Other available options include laparoscopic sheaths (Biogenesis, Bloomington, IL, USA) for universal small ruminant AI gun and ovine transcap guide with aspic sheath for mini straws (IMV Technologies, 61300 L'Aigle, France).

Semen processing equipment can include a microscope, a slide warmer, semen straw cutters, and a semen thawing unit. A generic microscope can be purchased for ~ \$300, or a semen specific microscope for ~ \$2,500. A slide warmer can be purchased for ~ \$200. Semen straw cutters are relatively inexpensive at \$15 each. A semen thawing unit can be purchased for ~ \$200. Cost of new and refurbished laparoscopic AI equipment is provided (Table 7).

**Table 7.** Cost of new and refurbished laparoscopic AI equipment

Equipment	New (\$)	Used/refurbished (\$)
Laparoscopy	7,000	2,500
Light source	7,000	1,400
Trocar/Canula	500	250
Video camera and screen	3,500	-
Air insufflation unit	8,500	300
Cradle	1,800	1,000
LAI gun	500-550	200
Semen processing	3,000	715

Costs associated with training (such as course fee, travel, hotel, etc.) and practicing on smaller flocks to gain proficiency are key factors in calculating breakeven costs. However, these factors are not included in this economic analysis because training costs and the time required to train an individual can vary substantially.

In general, a set of refurbished equipment can be purchased for ~ \$4,000. Fiber optic light sources and scopes with digital image abilities will increase the cost and are often not necessary. Buying new equipment or job-specific equipment can also increase the cost but may increase the lifespan of the equipment. Many companies sell LAI kits (e.g. 'Sheep LAI set' from MEDIT) with fiber optic light source, 0-degree scope, and 2 trocars and cannulas for \$2,300. These can be a viable choice for veterinarians who like to upgrade their kit or purchase new equipment guaranteed to be compatible.

Consumables are also needed to perform LAI. Consumables can include insemination pipettes and semen. Robertson insemination pipettes can be purchased in bundles and the cost is ~ \$20 or less per pipette. Semen cost is variable, depending on the breed and source.

**Breakeven cost assessment (refurbished equipment procurement with laparoscopic artificial insemination service)**

Breakeven point (Table 8) at which LAI service would become profitable was determined using current market prices, with the following assumptions.

Veterinarian made only 1 trip to inseminate required number of ewes over a 3-hour period, ~ 25 ewes/hour.

**Expenses**

- Used/refurbished equipment cost - \$4,000.00 -
- Technician - \$18.00/hour
- Trailer rental - \$15.00/hour
- Needle/syringe/blade/glue - \$3.00/ewe
- Sedation - \$0.50/ewe
- Synchronization drug cost (CIDR, PG600 and PGF<sub>2α</sub>) - \$19.00/ewe

**Table 8.** Economic analysis of breakeven (expense and profit) cost associated with procurement of refurbished laparoscope and laparoscopic artificial insemination service

Itemized list	Price (\$) per	Quantity	Cost (\$)	Revenue (\$)
Expense:				
Laparoscope	4,000.00	1	4,000.00	
Technicians	18.00/hour; 3 hours	2	108.00	
Trailer rental	15.00/hour; 3 hours	1	45.00	
Consumables/ewe				
Needle/Syringe/blade/glue	3.00	71	413.00	
Sedation (60 kg ewe)	0.50	71	35.50	
Synchronization (CIDR-S, PG600, PGF <sub>2α</sub> )	19.00	71	1349.00	
Total expenses:			<b>5,750.50</b>	
Charges				
LAI	80.00	71		5,680.00
Farm call	75.00	1		\$75.00
<b>Total Revenue</b>				<b>5,755.00</b>

Veterinary service revenue

- LAI service charge - \$80.00/ewe
- Farm Call - \$75.00/trip

Performing LAI in 71 ewes would breakeven the expenses needed to procure the refurbished laparoscopic equipment.

**Economic advantages of laparoscopic AI over natural and transcervical AI**

Economic advantage of LAI over TCAI and natural service was determined using a crude economic analysis (Table 9a, 9b, 9c) with following assumptions.

Assumptions used for laparoscopic artificial insemination:

LAI was performed in 10 ewes with 70% pregnancy rate and 1.6 lambs/ewe (show-quality lambs)

- Ram lamb: ewe lamb ratio 50:50
- Cost for frozen semen - \$ 100/insemination dose
- Feeding lambs to 90 lbs - \$ 52.50/lamb
- Technician - \$ 18.00/hour
- Trailer rental - \$ 15.00
- Needle/syringe/blade/glue - \$ 3.00/ewe
- Sedation - \$ 0.50/ewe
- Synchronization drug cost - \$ 19.00/ewe (CIDR, PG600 and PGF<sub>2α</sub>)
- Market price for ram lamb \$1,100/lamb
- Market price for ewe lamb \$1,000/lamb

Assumption used for transcervical artificial insemination:

- TCAI was performed in 10 ewes with 25% pregnancy rate and 1.6 lambs/ewe (show quality lambs)
- Ram lamb: ewe lamb ratio 50:50
- Cost for frozen semen \$100/insemination dose

- Feeding lambs to 90 lbs \$ 52.50
- Technician - \$ 18.00/hour
- Trailer rental - \$ 15.00
- Needle/syringe/blade/glue - \$ 3.00/ewe
- Sedation - \$ 0.50/ewe
- Synchronization drug cost - \$ 19.00/ewe (CIDR, PG600 and PGF<sub>2α</sub>)
- Market price for ram lamb \$1100
- Market price for ewe lamb \$1000

Assumptions for natural breeding:

- Natural breeding was performed in 10 ewes with 66% pregnancy rate and 1.6 lambs/ewe (show quality lambs)
- Ram lamb: ewe lamb ratio 50:50
- Ram value \$7500.00
- Feeding lambs to 90 lbs \$52.50/lamb
- Feeding ram for a year \$700.00/ram
- Synchronization drug cost \$19.00/ewe (CIDR, PG600 and PGF<sub>2α</sub>)
- Market price for ram lamb \$600/lamb
- Market price for ewe lamb \$600/lamb

Breakeven point at which LAI services become profitable was determined using current market prices (Table 8); analysis assumed that veterinarian made only 1 trip and inseminated 71 sheep in 3 hours.

Cost to benefit for LAI, TCAI, and natural breeding from a producer's standpoint are compared (Tables 9a-c). Though natural service and transcervical AI breeding programs produced ~ \$2,000 profit, laparoscopic AI produced ~ an additional \$6,800 monetary benefit.

In addition to monetary benefits, LAI has several others. Although there is a higher overhead cost associated with LAI over more conventional AI methods, it is still a

**Table 9a.** Economic analysis for profit estimation generated after implementing laparoscopic artificial insemination procedure (expenses and revenues considered, based on the assumptions listed\*)

Itemized List	Price (\$) Per	Quantity	Revenue (\$)	Cost (\$)	Profit (\$)
Expenses:					
Farm call	75.00	1 trip		75.00	
LAI service charge	80.00	10 ewes		800.00	
Cost of frozen semen	100.00	10 doses		1,000.00	
Synchronization drug	19.00	10 ewes		190.00	
Needle/syringe/blade/glue	3.00	10		30.00	
Sedation	0.50	10		5.00	
Trailer rental	15.00	1		15.00	
Technician	18.00/hour	1		18.00	
Feeding lambs to 90 lbs	(52.50)	11*		577.50	
*Total lambs (Pregnancy rate 70%; 1.6 lambs/ewe) - 11.2					
Profit:					
Sale of ram lambs	1,100.00	5	5,500.00		
Sale of ewe lambs	1,000.00	6	6,000.00		
<b>Total</b>			<b>11,500.00</b>	<b>2,710.50</b>	<b>8,789.50</b>

practical option for many veterinarians. The technical skills and equipment required for LAI are more substantial than TCAI but the benefits of LAI include bypassing the cervix, access to a deeper site of semen deposition, more efficient use of sperm, and higher conception rates translating to added value service delivered to clients. Frozen semen dose for LAI is  $20\text{-}25 \times 10^6$  sperm ( $300\text{-}400 \times 10^6$  and  $150\text{-}200 \times 10^6$  for transvaginal and transcervical AI, respectively).

Sexed semen option is available for sheep and is worth considering. However, it should be based on the producer's goals; it

is vital to perform a cost benefit analysis to assess the value of sexed semen in each specific scenario.

### Conclusion

Laparoscopic artificial insemination is a highly effective and proven method of artificial insemination that has several advantages over traditional insemination techniques. One of the primary benefits of LAI is its precision and ability to achieve higher success rates that translates into improved reproductive outcomes for livestock producers. Although there is an initial investment required to purchase the specialized equipment, the costs are relatively modest when

**Table 9b.** Economic analysis for profit estimation generated after implementing transcervical artificial insemination procedure (expenses and revenues considered, based on the assumptions listed\*)

Itemized List	Price (\$) Per	Quantity	Revenue (\$)	Cost (\$)	Profit (\$)
Expenses:					
Farm call	75.00	1 trip		75.00	
TAI service charge	60.00	10 ewes		600.00	
Cost of frozen semen	100.00	10 doses		1,000.00	
Synchronization drug	19.00	10 ewes		190.00	
Needle/syringe/blade/glue	3.00	10		30.00	
Sedation	0.50	10		5.00	
Trailer rental	15.00	1		15.00	
Technician	18.00/hour	1		18.00	
Feeding lambs to 90 lbs	(52.50)	4*		210.00	
*Total lambs (Pregnancy rate 25%; 1.6 lambs/ewe) - 4					
Profit:					
Sale of ram lambs	1,100.00	2	5,500.00		
Sale of ewe lambs	1,000.00	2	6,000.00		
<b>Total</b>			<b>4,200.00</b>	<b>2,143.00</b>	<b>2,057.00</b>

**Table 9c.** Economic analysis for profit estimation generated after implementing natural service breeding program (expenses and revenues considered, based on the assumptions listed\*)

Itemized List	Price (\$) Per	Quantity	Revenue (\$)	Cost (\$)	Profit (\$)
Expenses:					
Cost of the ram	7,500.00	1		7,500.00	
Feeding ram for 1 year	700.00	1		700.00	
Ram BSE	45.00	1		45.00	
Synchronization drug	19.00	10 ewes		190.00	
Feeding lambs to 90 lbs	(52.50)	11 *		577.50	
*Total lambs (66% pregnancy rate; 1.6 lambs/ewe) 11					
Profit:					
Ram lambs	600.00	5	3,000.00		
Ewe lambs	500.00	6	3,000.00		
Sale price of ram after 1 year	5,000.00	1	5,000.00		
<b>Total</b>			<b>11,000.00</b>	<b>(9,012.50)</b>	<b>1,987.50</b>

compared to the long-term advantages. Additionally, the overhead costs associated with this service are minimal, making it an economically viable option for veterinary practices. For practitioners who already have a sufficient client base, the equipment expenses can often be recouped within just 1 breeding season, providing a strong return on investment.

However, several factors must be carefully considered to ensure the success of artificial insemination in sheep. These include the quality of the semen used, proper storage conditions, animals' fertility level, effective management practices, and strict adherence to scheduling protocols. Additionally, producer compliance with recommended procedures and the timing of insemination relative to the breeding season are all critical to achieving the desired outcomes. It is essential that these factors are monitored and controlled to minimize risks and optimize results. Any variation or oversight in these areas can significantly impact the success rates of the procedure.

The economic benefits of laparoscopic artificial insemination are substantial, especially to veterinarians who can provide this service to a broader client base. By increasing conception rates and reducing the need for costly repeated matings, LAI can ultimately save producers money while also contributing to higher overall productivity. For veterinary practices, adding LAI procedures provides a valuable opportunity to differentiate themselves in a competitive market. Effectively communicating these benefits to clients is crucial, as it helps to underscore the advantages of the service and fosters trust between practitioners and producers. LAI not only enhances the economic viability of a practice but also adds a layer of expertise and specialization, further enriching the practice's reputation and service provided.

### Conflict of interest

None to report.

### Author contribution

Authors equally contributed to this work.

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