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Review Article

CURRENT STATUS OF LAPAROSCOPIC ARTIFICIAL INSEMINATION IN SHEEP BREEDING PROGRAM AND FACTORS AFFECTING ITS APPLICATION: A REVIEW

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ABSTRACT
Artificial insemination (AI) is used to accelerate the genetic gain and improve reproductive performances in farm animals but AI in sheep is currently limited by the poor fertility obtained following non-surgical intracervical insemination of frozen-thawed semen. Therefore, this review emphasized on the current status of laparoscopic artificial insemination (LAI) in sheep breeding program and factors affecting its application. Even though, there are many factors which limit the application of LAI in sheep breeding program, this review was focused on the breed type, the complex structure of cervix and time of insemination. Based on this review, different studies indicated that the pregnancy rate after insemination affected the fertility of different sheep breeds such as the Australian merino, Suffolk and Finnish landrace. The time of AI is especially important in the case of estrus synchronization. The best time of insemination could be 46 to 63 hours after the pessary removal. So as the median time of ovulation was 56-60 h, indicating that conception rates were higher when insemination took place shortly after ovulation. In general, AI in sheep breeding has limitation due to the complex cervical structure of the ewes. Therefore, LAI is the only method tackle this problem in sheep breeding by depositing the directly semen in the horn of the ewes cervix surgically.

Keywords: *Artificial insemination, laparoscopic artificial insemination, breed, pregnancy rate*

INTRODUCTION
Assisted reproductive technologies (ART) are utilized in animal reproduction to promote efficient use of germplasm for improvement of genetic value of production animals. It has also enabled the use of sophisticated data analysis procedures to identify animals of superior performance. The availability of an efficient sheep artificial insemination (AI) service would yield similar benefits and would greatly enhance the scope for pedigree and commercial breeders to respond positively and effectively to consumer demands. The widespread use of AI and the realization of its full potential depend essentially on the use of frozen semen, and thus, on the availability of techniques that result in acceptable fertility (Donovan *et al.*, 2001).

ART in farm animals are used to cater the needs for higher productivity and better quality of products. In sheep production, the use of AI has enabled the rapid introduction of valuable genes that improved production traits and prevented disease transmission but the implementation of AI in sheep globally, is relatively limited (Faigl *et al.*, 2012).

AI in sheep has been poorly implemented and is carried out mainly with chilled semen because of the low fertility results obtained when using frozen-thawed semen (Salamon *et al.*, 1995).

Laparoscopic artificial insemination (LAI) is an advanced ART that enables such deep intrauterine deposition of semen and intracervical artificial insemination (ICAI) that helps bypass the physical barriers of the caudal reproductive tract in sheep. The pregnancy rates using frozen semen deposited intrauterine via laparoscopy had been yielded higher pregnancy rates (60-80%) consistently when compared to vaginal and trans-cervical artificial insemination (TCAI) methods (McKusickn *et al*., 1998). In addition to

higher pregnancy rates, it is possible to use lower concentrations of spermatozoa per breeding thus leading to more animals bred per ejaculate. The average dose required for breeding a ewe using frozen semen can be as less as 20-25 million live spermatozoa when compared to higher doses required via the vaginal (400 million live spermatozoa) and TCAI (100-200 million live spermatozoa) (Anel *et al.,* 2003). Thus, a single entire frozen ejaculate can be used to inseminate as many as 50 to 100 ewes, thus leading to more efficient use of semen. Because of, ewes/does bred with frozen or processed semen typically have a lower pregnancy rate when bred via conventional methods such as vaginal (VAI) and TCAI techniques due to anatomical nature of the ewes (Fair *et al.,* 2004). The number of reports in which TCAI deposition of semen has been achieved is relatively low and there are concerns about the potential trauma involved. Thus, while laparoscopic AI is a very effective technique, the costs involved in terms of expertise, labour and equipment are high, limiting its use.

A better understanding of the reasons for the generally low conception rate achieved following cervical deposition of frozen–thawed semen is still an important objective towards the goal of establishing a cost-effective and widely applicable AI procedure for achieving acceptable pregnancy rates in sheep (Faira *et al.,* 2012, Paulenz *et al.,* 2004). The main disadvantage of providing LAI service to producers has been the high equipment cost and the relatively steep curve of surgical expertise required to perform the procedure safely. However, with the availability of newer and more portable laparoscopy equipment, it is now possible to offer these services cost-effectively at a hospital and field setting.

The fertilization failure is equally frequent in ewes bred naturally or artificially inseminated and appears to be due to faulty transport of spermatozoa through the cervix (Boland *et al.,* 1983). This problem can be overcome by intra-uterine deposition of semen through a surgical procedure in superovulated ewes or by laparoscopic insemination (Ishwar *et al.,* 1996). The impact of AI in the sheep industry has not been fully realized in its ability to improve production (e.g., carcass quality, wool, and milk). However, in recent years, emphasis on genetic improvement has moved ahead of methods of former years of selection on visual appraisal and commonly show ring for various popular breeds of sheep.

Factors affecting laparoscopic artificial insemination in sheep

Factors influencing the success of LAI of sheep have several factors that could modify the effectiveness of LAI and some of them are the fertility and dose of the semen, proper handling of the semen, the proper site of semen deposition and semen transport, the use of fresh, cooled, chilled, frozen semen, time of insemination after estrus synchronization, the anatomical structure of the ewe's cervix, breeds, synchronization protocol, technician and other management factors but in this review only focus the breeds, the anatomical structures of ewes and the times of insemination, Laparoscopy is a technique for examination of the abdominal cavity and its contents. It requires insertion of a cannula/trocar through the abdominal wall, distension of the abdominal cavity with sterile air or co_2 and visual examination of the abdomen's contents with an illuminated telescope. Therefore, LAI is an advanced technology that enables such deep deposition of semen in sheep breeding programs that helps bypass the physical barriers of the anatomical structures of ewe's reproductive organs.

Anatomical structure of the ewe's cervix

The ewe's cervix has a distinctive, highly complex structural arrangement that precludes easy trans cervical passage and intrauterine deposition using conventional. It is a very long and narrow fibrous organ comprised of mainly connective tissue with an outer serosal layer and inner luminal epithelium. The inner lumen adopts a tortuous quality due to the presence of characteristic funnel-shaped; caudally facing rings that project into the lumen along the length of the cervix (Halbert *et al.,* 1990 and Kershaw *et al.,* 1990). This causes a drastic narrowing of the lumen, which is the most constricted at the first two caudal rings. The ostium of each cervical ring is ~2.7 mm in diameter and is not concentrically aligned with that of adjacent rings. As a result, advancing a pipette through the cervix is problematic due to the difficulty in locating the opening of each successive, eccentrically positioned ring, in addition to a very small luminal

diameter. There is a great deal of variability in cervical morphological characteristics between breeds and individual ewes, including the length (5-10 cm), a number of rings (3-7), ring ostium diameter (1-3 mm), and the distance between rings 3-5 cm. It has also been suggested that the number of folds presents increases with age and parity (Halbert *et al.*, 1990; Naqvi, 1998). On the other hand The mean length of the cervical channel has been described as, 6.5, 5.5 and 6.7 cm (Fukui and Roberts 1978, Halbert *et al.*, 1990; Hemmoda *et al.*, 2000) respectively and the length ranges from 5.7 to 10 cm (Abusineina, 1969) illustrating the high variability between individuals according to the types of breed. Therefore, designing a customized AI catheter for sheep has been particularly challenging (Anel *et al.*, 2006).

It is these cervical rings that present the major barrier to TCAI as they project into the lumen and the second and third rings are frequently out of alignment with the first, resulting in the inseminating pipette being misdirected away from the central lumen Furthermore, the highly constricted external aperture of the cervix, or cervical that extends into the vagina, is immediately surrounded and, in many cases, completely obscured by vaginal folds of fibrous tissue (Kershaw *et al.*, 1990). The configurations manifested by the external of the cervix and surrounding vaginal folds are also highly variable between ewes.

a b c

Figure 1a-c. a. Laparoscopic view of follicle on sheep ovary, **b.** Laparoscopic view of a sheep uterus in estrus and, **c.** Morphology of sheep cervix during the periovulatory period of estrus (Leethongdee, 2010)

Therefore, the structure of ewe's cervix is difficult to inseminate trans cervical artificial insemination methods due to the presence of many rings and the length of the cervix so as surgical LAI in ewes are preferable to put the semen into the horn of uterus easily.

The success of TCAI in ewes is highly dependent on the anatomy of the cervical lumen and the stage of the estrous cycle. Therefore, Laparoscopic insemination in the sheep breeding industry has a great value and one of the recent breeding technologies in different countries and research institute areas to solve these anatomical problems.

The current status of laparoscopic artificial insemination on sheep in different countries

LAI of sheep the pregnancy rates using frozen semen deposited intra-uterine via laparoscopy have yielded higher pregnancy rates (60-80%) consistently when compared to vaginal and TCAI methods (McKusic *et al.*, 1998). In addition to higher pregnancy rates, it is possible to use lower concentrations of spermatozoa per breeding thus leading to more animals bred per ejaculate. The average dose required for breeding a ewe using frozen semen can be as less as 20-25 million live spermatozoa, when compared to higher doses required via the vaginal (400 million live spermatozoa) and TCAI (100-200 million live spermatozoa)

(Anel *et al.*, 2003). As shown below in table 1 the different author has reported the efficiency rates of laparoscopic insemination. Though LAI of 7 ewes from the total number of 9 ewes was conceived it, and according to the study it was important to estimate pregnancy rate of this method and to know the capability of laparoscope in insemination of ewes artificially, so that; the results showed percentage of 71.4% of pregnancy in ewes inseminated Laparoscopically (Abdalbari *et al.*, 2012). But also many workers report variable and generally poor pregnancy rates following cervical insemination with frozen-thawed semen, an exception is Norway the conception rates of 60% are reported at field level (Olesen, 1993). Thus a central task was to determine if similar success could be achieved in Ireland by adopting the freezing and insemination procedures used in Norway. However, breeds used in Norway are quite different from those found in Ireland and insemination is to a natural estrus. In Ireland, this would be impractical and any serious thought of applying AI here would require set-time AI to a synchronized estrus. It was necessary to determine if the good conception rates achieved in Norway are due to inherent Norwegian factors/practices and/or the breeds of sheep involved (Donovan *et al.*, 1997).

Even though for this procedure, expensive equipment and a well-trained team are required. Fertility results among practitioners are still inconsistent because many factors contribute to the success of any AI program. In this sense, Anel *et al.*, (2005) was analyzed 44 488 inseminations in the Churra breed (Spanish milk breed), showing a great difference between vaginal and cervical inseminations and laparoscopic inseminations with regard to fertility, as well between farms, years, seasons, age of ewes, lambing-AI interval, ram and technician. Therefore, conception rate for LAI was 44.9% and for vaginal insemination 31.6% ($p < 0.001$). On similar studies the author, Hill et al., 1998 reported that the conception rate was 48-72% from Rambouillet ewes breed from USA. Moreover, in Italy the conception rate wat record 70 from Baracel ewe breeds (Pau,*et al.*, 2020). Anyway, laparoscopic artificial insemination (LAI) is now being used in the sheep industry around the world as a standard method to extend the use of superior rams. It offers the farmer an opportunity to maximize the reproductive potential of sheep. The primary economic benefit to the sheep producer is a rapid infusion of valuable genetic traits into the flock (Luther, 2008). Semen trading between countries is only possible with frozen semen and thus using LAI is of paramount importance.

Table 1: The current status of laparoscopic artificial insemination in different sheep breeds in different countries

Synchronization status	**Sheep breed**	**Country**	**No of sample**	**Frequency occurrence Conception rate**	**Semen used**	**Reference**
Synchronized	Churra breed	Spain	44, 488	44.9%	Frozen-thawed sperm	Anel *et al.*, 2005
Synchronized	Arabi	Iraqi	9	71.4 %	frozen semen	Abdalbari *et al.*, 2012
Synchronized	Finnish Landrace	Ireland	297	61%	frozen semen	Olesen, 1993; Donovan, 1997
Synchronized	Rambouillet	USA	93	48-72 %	frozen semen	Hill *et al.*, 1998
synchronized	Baracel	Italy	40	70	Frozen semen	Pau *et al.*, 2020

Generally, the fertility rate of the breed has an effect on both the pregnancy and conception rate of the sheep breeding program after AI so as the breeder conceder different parameters in different countries and

research institutes in different years. Therefore, the LAI technique can enhance the fertility rates of ewes in sheep breeding programs through practices by considering those factors.

Effect of ewe breed on pregnancy rate

In many publications, the ewe breed has been found to have a large effect on the pregnancy rate after artificial insemination. According to Hill *et al.*, (1998), the wool type (strong wool-67.6%; fine wool-71.7%; fine medium and medium 73%) of Australian Merino affected the average pregnancy rate. In a Greek study (Karagiannidis *et al.*, 2001), on the similar studies in Irish Suffolk ewes was much lower (12%) than that of Finnish landrace (65%) (Donovan *et al.*, 2001), while the breed of the ram also had a significant effect on prolificacy after AI (Perkins et al., 1996; Donovan et al., 2001; Anel *et al.*, 2005). The conception rates of ewes have a significant difference between them as shown in table 2. However, the other author has reported the major differences among ewe breeds with respect to pregnancy rate to AI with frozen-thawed semen, regardless of the source of that semen. Thus, the average pregnancy rate over the set of AI experiments was as follows:-Finnish Landrace ewes 61%, Belclare ewes 44% ,Belclare x Scottish Blackface ewes 44% ,Texel ewes 31% and Suffolk ewes 12% with laparoscopic insemination (Donovan, 2001).

Table 2: The pregnancy rate (%) for ewes inseminated with fresh or frozen-thawed semen

Breed	**Pregnancy rate (%)**	**Semen used**	**Reference**
Australian Merino	strong wool 67.6;	Frozen-thawed semen	Hill *et al.*, 1998
	fine wool 71.7	Frozen-thawed semen	
	Fine medium and medium 73	Frozen-thawed semen	
Suffolk	12	Frozen-thawed semen	Donovan *et al*., 2001
Finnish landrace	65	Frozen-thawed semen	Donovan *et al.*, 2001
Indigenous Greek ewes	44.1	Frozen-thawed semen	Pappa *et al*., 1999

As shown in the above table 2 the results for the breed confirmed a significance of ewe breed ($p< 0.05$) as a major factor determining the success of LAI with frozen-thawed semen. The pregnancy rate for the different breeds is shown in Table 2. Australian Merino ewes had the highest pregnancy rate and Suffolk ewes had the lowest pregnancy rate. Finnish landrace ewes had similar results which were significantly better than Suffolk crosses, whereas indigenous Greek ewes which were intermediate. There was significate effect due to breed types on the pregnancy rate whereas there was no significant effect due to the source of semen but differences among individual rams were statistically significant effects on the result. The breed is consistently found to have a very large effect on pregnancy rate in the studies reported here. The range in values was from 12% for Suffolk ewes to 73% for Australia merino ewes. So LAI is an excellent technique to get the birth rate close to 100% and improve the genetic value of the flock of sheep breed. Even if the percentage of pregnancy rate after LAI in sheep is significantly affected by different parameters. LAI technique is preferable in sheep breeding programs.

Effect of insemination time on conception rate

Laparoscopic AI is almost always used in conjunction with the synchronization of estrus and possibly also with stimulation of ovulation. Since only small numbers of animals are usually inseminated, at least about cervical AI programs, the synchronization is an essential component of a LAI program so that all females can be inseminated over a short period. The time of insemination was much more critical for frozen-thawed semen than for fresh semen. The author, Maxwell (1986a) was determined that the optimum time for intrauterine insemination of ewes treated with a progestogen sponge and 400 IUC.

Pregnant mare serum gonadotropin (PMSG) was 60-72 h after sponge withdrawal; the median time of ovulation was 56-60 h, indicating that lambing rates were higher when insemination took place shortly after ovulation. The time of AI is especially important in the case of estrus synchronization. On similar studies the best time of insemination could be 46 (Fernandez-Abella et al., 2003), 48 to 72 (Karagiannidis et al., 2001), and 58 to 63 (Donovan et al., 2001) hours after the pessary removal. The lower pregnancy rates following insemination of frozen-thawed semen in pure-bred lowland breeds, most especially the Suffolk, may be related to the within-breed spread of ovulation time. This problem is compounded by the severely reduced survival time of frozen-thawed ram semen in the female reproductive tract. Acceptable pregnancy rates may require double insemination to overcome the problem of high variation in the timing of ovulation. Though as shown in table 3 early insemination times is has a significant effect on the pregnancy rate of ewes in the sheep breeding programs.

Tabele 3: Effects of time insmination on pregrance rate (%)

Insmination time (hr)	Pregarncy rate (%)	References
57	34	Donovan *et al.*, (2001)
63	33	
57 and 63	43	

Therefore, insemination the early time of insemination is good and preferable for pregnancy rate of the sheep breeding program. You should have to inseminate on the appropriate time after the removal of the hormone that are used to for synchronization purpose. Another author also reported the median time of ovulation was 56-60 h, indicating that lambing rates were higher when insemination took place shortly after ovulation to get a higher pregnancy rate of ewes. So intrauterine insemination is especially effective in overcoming the fertilization failure of donors exhibiting high ovulation rates. In sheep, intrauterine laparoscopic insemination should be carried out in the middle of the estrous period (Walker *et al.*, 1986).

CONCLUSION

LAI is a minimally invasive procedure where ram semen is deposited directly into the uterine horns of the ewe. The success of Surgical AI in ewes is highly dependent on the anatomy of the cervical lumen and the stage of the o-estrous cycle, even if the procedure requires surgical skill and costly equipment. Depositing frozen-thawed semen at the cervix results in low conception rates. The inability to pass an insemination pipette through the cervix has prevented artificial insemination in sheep from becoming a standard breeding method as in the cattle breeding. In general, LAI is the only method to enhance the offspring of sheep. The difficulties in technique may be solved by training. In conclusion, the percentage of pregnancy after AI in sheep is significantly affected by different parameters such as the breed type, the insemination time after synchronization and the anatomical structures of ewe's cervix. Even if AI technique is preferable in sheep breeding programs but still the pregnancy rate of the ewes is not achieved to 100% due to different reasons. In order to overcome these problems, the LAI technique is one of the recent technologies to solve the limitations of AI in sheep. LAI is an excellent technology to get the birth rate close to 100%. We have chosen to focus on LAI techniques to obtain and increase in overall fertility rates sheep breeding program.

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