

STUDIES ON LAPAROSCOPIC INTRAUTERINE INSEMINATION OF BARKI EWES (USING DIFFERENT INSEMINATION DOSES) AS COMPARED WITH CERVICAL INSEMINATION

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ABSTRACT

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The present study aimed to investigate the efficacy of laparoscopic intrauterine insemination as compared with cervical insemination in Barki ewes as well as to set the optimum number of spermatozoa per laparoscopic insemination. A total of 33 oestrus-synchronized Barki ewes were divided into four groups, the first three groups were inseminated laparoscopically with either 10×10^7 (group 1), 20×10^7 (group 2) or 40×10^7 sperm/dose (group 3). The 4th group was cervically inseminated. The results showed that lambing rate after laparoscopic intrauterine insemination using 20×10^7 spermatozoa (75.00%) was superior to either laparoscopic insemination using 10 or 40×10^7 spermatozoa or cervical insemination using 80×10^7 spermatozoa (37.50%, 62.50 and 55.56%, respectively). Conclusively, intrauterine insemination using the technique of laparoscopy is a relatively simple, field applicable and convenient mean of achieving high lambing rates. Furthermore, 20×10^7 motile spermatozoa is the recommended laparoscopic insemination dose in Barki ewes.

Key words: *Laparoscopic, Intrauterine insemination, Barki ewes.*

INTRODUCTION

Artificial insemination has become an important adjunct for breeding certain domestic species because of its great advantage for exploiting the genetic potential of superior sires. It has been known for quite long time that cervical insemination in sheep though commercially widely used, does not render satisfactory fertilization rate (Amiridis and Cseh, 2012). The cervical canal of the ewe has a convoluted and tortuous structure, reflecting the presence of 5–7 annular folds or cervical rings (Kershaw *et al.*, 2005; Kaabi *et al.*, 2006) that prevent trans-cervical intrauterine insemination (Kaabi *et al.*, 2006).

Laparoscopic approach for intrauterine semen deposition is an easy technique, however, giving acceptable fertilization rates in estrous synchronized ewes (Lymberopoulos *et al.*, 2001; Naqvi *et al.*, 2001;

Evans *et al.*, 2002; Hiwasa *et al.*, 2009). The advantage of laparoscopic insemination is that the semen is deposited closer to the site of fertilization. Deep uterine insemination has been shown to be advantageous in several domestic species, such as sheep (Salamon and Maxwell, 1995; Wulster-Radcliffe *et al.*, 2004), goats (Ritar and Salamon, 1983; Moore *et al.*, 1988), cattle (Lopez-Gatius, 2000; Verberckmoes *et al.*, 2004), horses (Morris and Allen, 2002), and pigs (Martinez *et al.*, 2002; Rath, 2002; Watson and Behan, 2003), especially when sperm numbers are limited or sperm quality is suboptimal.

The site of insemination of ram semen has a major effect on pregnancy rate in sheep, with greater rates achieved following laparoscopic AI than following either transcervical (Wulster-Radcliffe *et al.*, 2004) or cervical insemination (Fair *et al.*, 2005). Using laparoscopy, the “cervical barrier” problem has been overcome and satisfactory fertility rate has been

achieved by significant reduction in the number of spermatozoa per insemination (from 200-300 to 1-10 millions, or less for sex-sorted semen, Salamon and Maxwell, 2000).

The major objectives of this study were; to compare the pregnancy and lambing rates of ewes inseminated either cervically or by intrauterine laparoscopic insemination and to determine the minimal sperm dose per intrauterine insemination in order to maximize the genetic diffusion of males, without decreasing AI success.

MATERIALS and METHODS

Animals and treatment:

Thirty six mature, clinically healthy Barki sheep (3 rams and 33 ewes), aged 2.0 -2.5 years were assigned to the study. Animals were kept in the experimental farm of the Animal Reproduction Research Institute (ARRI), and they were divided into four groups, the first three groups (8 ewes in each) were inseminated laparoscopically with either 10×10^7 (group 1), 20×10^7 (group 2) or 40×10^7 sperm/dose (group 3). The last group (group 4 containing 9 ewes) was transcervically inseminated with 80×10^7 sperm/dose (Gordon, 1997).

Collection of semen:

Semen was collected from trained rams with an artificial vagina that was adjusted to a proper condition, diluted at 30°C with Tris-based diluent to provide a sperm concentration of 80×10^7 /ml, cooled slowly and kept at 16°C for up to 6 h (Langford *et al.*, 1979). Only rams with at least 70% motile spermatozoa and good progressive motility were used. Just before insemination, dilutions were made at 16°C using Tris-based diluent to provide concentrations of 80, 40, 20 and 10×10^7 /ml.

Synchronization of estrus:

The estrous cycles were synchronized using CIDR's (EAZI-BREED, CIDR®, New Zealand), inserted for a period of 14 days (Sirjani *et al.*, 2012). eCG (250 IU; Folligon; Germany) intramuscularly injected to

ewes at the time of CIDR removal. The laparoscopic and cervical inseminations were performed on the 58th hour after removal of CIDR (Bonev *et al.*, 2005).

Laparoscopic artificial insemination:

Laparoscopic procedures were done using Wolf Laparoscope (Wolf Co., Germany) of 5 mm diameter, 33 cm length and 0° scope viewing angle. Automatic insufflator was used to deliver the CO₂ intraperitoneally (pressure 10 mmHg). Laparoscopic insemination, in details was described by Toni *et al.* (2012). Briefly, ewes were fasted and restricted access to water at least for 16 hours before laparoscopy, and injected intravenously with xylazine hydrochloride (Xylaject 2%, Adwia) at a dose rate of 0.05mg/kg b.w. Local anesthetic (Lidocaine 2%) was injected 10 minutes before the procedure was performed. The ewe was then placed in a laparoscopy cradle. The abdominal region was surgically prepared by shearing the wool and disinfecting the skin. Using the cradle, the ewe was positioned in a supine head-down (Trendelenburg) position to an approximate angle of 30° (Fig 1). A scalpel blade was used to make a small skin incision in order to facilitate trocar penetration (Fig 2). The trocars and cannulae for introducing laparoscope and insemination pipette were inserted 7-10 cm ventral to the udder and 5-10 cm on each side of the midline (*linea alba*). The 5-mm Verus needle that was connected with the CO₂ is first introduced and the abdomen was slightly inflated to reduce the chance of injury to organs. Insertions of the first trocar and cannula should be well controlled and the sharp trocar was withdrawn as soon as the abdominal wall has been penetrated. The blunt cannula was pushed well into the abdomen, while the second trocar and cannula were inserted after inflation with CO₂. Endoscope and AI instrument went through the cannulae (Fig 3) and the uterus was located and fixed using the grasping forceps just ventral to the urinary bladder. Semen was deposited in the lumen of each uterine horn approximately halfway between the uterine bifurcation and the utero-tubal junction (Fig. 4). Instruments were withdrawn and putted into disinfectants between each animal. An antibiotic spray was applied to the wounds before it was sutured. The sutures were removed 7 days later.



Fig. 1: Positioning of the animal in a supine head-down position for laparoscopy



Fig. 2: A small skin incision in order to facilitate trocar penetration



Fig. 3: Endoscope went through the cannulae for visualization of uterus

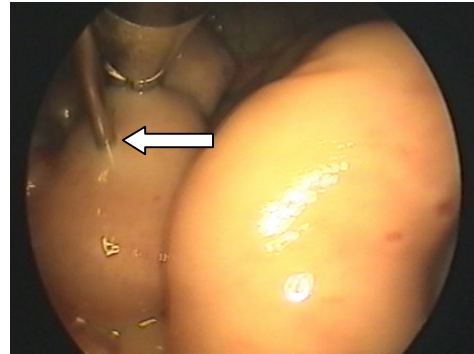


Fig. 4: The recommended site of puncture for semen deposition is the major curvature of the uterine horn (arrow).

Cervical artificial insemination:

The cervix was located, via a speculum fitted with a light source. The cervix of the ewe is convoluted in structure and does not dilate during oestrus. As a result it was generally only possible to deposit the semen (80×10^7 sperm) in the first fold of the cervix.

Pregnancy diagnosis:

Pregnancy rate (pregnant ewes/treated ewes ratio) was determined 35 days after AI by trans-abdominal ultrasonography examination using an Exagyne ECM, France) machine provided with a 6.5 MHz linear probe. Pregnancy loss in ewes was calculated as the number of ewes pregnant at Day 35 – the number of lambed ewes / number of ewes pregnant at Day 35.

Statistical analysis:

The recorded data among all treatment groups was analyzed by Chi square analysis. The level of significance was observed at 5% (Snedecor and Cochran, 1989).

RESULTS

Regarding the results of laparoscopic intrauterine insemination (table 1), the insemination dose of 20×10^7 spermatozoa (group 2) and 40×10^7 spermatozoa (group 3) resulted in the same pregnancy rate at 35 days post-insemination (75.00% for each). However, insemination of 20×10^7 spermatozoa resulted in significantly ($P \leq 0.05$) higher lambing rate when compared with using 10×10^7 and 40×10^7 spermatozoa (75.00% vs. 37.50 and 62.50%, respectively). On the other hand, ewes of group 1 had the significantly higher rate of pregnancy loss (40.00%) when compared with rates of groups 2 and 3 (0.00% and 16.67%, respectively).

The results presented in table (1) revealed that, laparoscopic intrauterine insemination using optimum insemination dose (20×10^7 spermatozoa, group 2) was superior to cervical insemination using 80×10^7 spermatozoa (group 4) in terms of pregnancy rate (75.00% vs. 55.56%, respectively) and lambing rate (75.00% vs. 55.56%, respectively).

Table 1: Pregnancy and lambing rates of ewes after cervical and laparoscopic intrauterine insemination using different insemination doses.

Method of Insemination	Laparoscopic intrauterine Insemination			Cervical Insemination
	GP1	GP2	GP3	GP4
Treatment Groups				
Insemination dose (sperm)	10 x 10 ⁷	20 x 10 ⁷	40 x 10 ⁷	80 x 10 ⁷
No. of ewes inseminated	8	8	8	9
No. of pregnant ewes (%)	5 (62.50) ^b	6 (75.00) ^a	6 (75.00) ^a	5 (55.56) ^c
Lambing rate (%)	3 (37.50) ^d	6 (75.00) ^a	5 (62.50) ^b	5 (55.56) ^c
Pregnancy loss (%)	2 (40.00) ^a	0 (0.00) ^c	1 (16.67) ^b	0 (0.00) ^c

Values with different superscripts in the same raw differs significantly at $P < 0.05$

DISCUSSION

In the current study, the lambing rate after laparoscopic insemination using 20 x 10⁷ spermatozoa (group 2) was significantly ($P \leq 0.05$) higher than that after cervical insemination using 80 x 10⁷ spermatozoa (75.00% vs. 55.56%, respectively). Similarly, Rojero *et al.* (2009) concluded that middle fertility rate of 43.7% resulting from cervical insemination in ewes can be considered as acceptable, but it is no possible to obtain similar fertility rate (75.00%) as with laparoscopic intrauterine insemination. Artificial insemination techniques have been considered in many previous studies. According to several authors (Armstrong and Evans, 1984; Rodriguez *et al.*, 1988; Correa *et al.*, 1994; Byrne *et al.*, 2000; Romano, 2013), laparoscopic insemination ensures significantly higher parturition rates than trans-cervical insemination, despite the fact that relatively lower numbers of spermatozoa are used. This difference in fertility can be explained by the fact that the sheep cervix has a very high structural complexity, preventing deep cervical insemination (Halbert *et al.*, 1990; Kaabi, 2002). Laparoscopic insemination allows this barrier to be bypassed, improving fertility even with lower quality spermatozoa (Salamon and Maxwell, 2000; Naqvi *et al.*, 2001).

The lambing rate of 75.00% achieved after laparoscopic intrauterine insemination in present study coincided with the same rates (75.00%) reported by McKelvey *et al.* (1985) and Rojero *et al.* (2009), and higher than lambing rates of 48.00%, 71.10%, 43.90%, 72.70%, 60.00%, 71.00% and 71.40% reported by Windsor *et al.* (1994), Hill *et al.* (1998), McKusick *et al.* (2000); Paulenz *et al.* (2005), Toni *et al.*, 2012, Al-Wataar (2009) and Alfaris *et al.* (2012), respectively. Sayre and Lewis (1997) reported

a higher lambing rate (92.50%) after intrauterine insemination. Concerning the cervical insemination, the lambing rate of 55.56% achieved after cervical insemination in this study was similar to that (57.00%) reported by Ghalsasi and Nimbkar (1996), and lower than lambing rates of 68.60%, 78.00%, 69.00%, 67.00%, 65.75% and 60.00% reported by Lightfoot and Salamon (1970), Langford *et al.* (1979), Tervit *et al.* (1984), McKelvey *et al.* (1985), Donovan *et al.* (2000), Nour *et al.* (2010), respectively, and higher than lambing rates of 43.70% and 50.00% reported by Rojero *et al.* (2009) and Al-Wataar (2009), respectively. The fertility rates following cervical and laparoscopic insemination all vary with the insemination technique used as well as with the farm, age, male, number of insemination per ewe, lambing-insemination interval, technician, flock and management conditions (Anel *et al.*, 2005; Paulenz *et al.*, 2005).

In sheep, the numbers of spermatozoa used by intrauterine insemination were reported to be 80 million (Windsor *et al.*, 1994), 150 million (Halbert *et al.*, 1990; Buckrell *et al.*, 1994), 200 million, (Lawrence, 1985; Husein *et al.*, 1998a) and 400 million (Smith *et al.*, 1995; Husein *et al.*, 1998b). In general, the numbers of spermatozoa used for intrauterine insemination are higher than the recommended numbers used for trans-cervical artificial insemination (Maxwell and Hewitt, 1986; Ritar, 1993; Romano, 2013). In the current study, a low intrauterine insemination dose (10x10⁷) resulted in decreased lambing rate (37.50%), whereas a higher insemination dose (20x10⁷) increased lambing rate (75.00%) and this result was in agreement with other researches (Maxwell and Salamon, 1993; Martin and Watson, 1976; Emsen *et al.*, 2011). Higher dose of spermatozoa (40x10⁷) was not recorded with significant increase in lambing rate (62.50%). This

result came in accordance with the findings of Emsen *et al.* (2011). Thus, it can be recommended that the minimum necessary for laparoscopic artificial insemination in Barki ewes is 20×10^7 motile spermatozoa. Similarly, Milczewski *et al.* (2000) recommended that higher pregnancy rates (69.56%) could be obtained with at least 25×10^7 spermatozoa per dose in intrauterine inseminations of ewes. On the other hand, Leethongdee (2010) recommended a minimum number of 40×10^6 spermatozoa per laparoscopic insemination. Also, Evans and Maxwell (1987) recommend a minimum dose of only 20×10^6 motile sperm while there are several reports of acceptable fertility (> 50%) using doses as low as 5×10^6 (Eppleston *et al.*, 1986) and 10×10^6 (Salamon *et al.*, 1985) motile spermatozoa. Furthermore, acceptable levels of fertility were achieved after low-dose insemination using flow cytometrically sorted ram sperm at a dose of 1×10^6 motile sperm per ewe (de Graaf *et al.*, 2007).

In conclusion, as compared to cervical insemination, intrauterine insemination using the technique of laparoscopy is a relatively simple and convenient mean of achieving high lambing rates in Barki ewes. The recommended insemination dose for laparoscopic artificial insemination of Barki ewes was 20×10^7 motile spermatozoa.

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دراسة مقارنة بين تلقيح النعاج البرقي بالمنظار داخل الرحم (باستخدام جرعات مختلفة من السائل المنوي) وداخل عنق الرحم

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يهدف هذا البحث إلى دراسة كفاءة تلقيح النعاج البرقي داخل الرحم بالمنظار مقارنة بالتلقيح بعنق الرحم بالإضافة لمعرفة العدد الأمثل من الحيوانات المنوية للتلقيح داخل الرحم. تم إجراء هذا البحث باستخدام ٣٣ نعجة برقي بعد عمل تزامن شبقى لها وتم تقسيمهن إلى أربع مجموعات ولقحت المجموعات الثلاثة الأولى منها داخل الرحم بالمنظار باستخدام ١٠^v X ١٠ (المجموعة الأولى) و ٢٠ X ١٠^v (المجموعة الثانية) و ٤٠ X ١٠^v حيوان منوى (المجموعة الثالثة) بينما لقحت نعاج المجموعة الرابعة إصطناعياً داخل عنق الرحم. وقد أوضحت النتائج أن معدل الولادة للمجموعة الثانية (٧٥.٠٠%) كان أعلى من معدلات الولادة في المجموعة الأولى والثالثة والرابعة (٣٧.٥٠%، ٦٢.٥٠%، ٥٥.٥٦%، على الترتيب). يستنتج من هذا البحث أن حقن السائل المنوي للكباش داخل الرحم عن طريق المنظار البطني يعتبر تقنية سهلة التطبيق تحت ظروف الحقل لتحقيق معدل مرتفع من الولادات وتوصى نتائج البحث بأفضلية استخدام ١٠^v X ٢٠ حيوان منوى كجرعة مثلى لتلقيح النعاج البرقي باستخدام هذه التقنية.