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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 x 10^7 (group 1), 20 x 10^7 (group 2) or 40 x 10^7 sperm/dose (group 3). The 4th group was cervically inseminated. The results showed that lambing rate after laparoscopic intrauterine insemination using 20 x 10^7 spermatozoa (75.00%) was superior to either laparoscopic insemination using 10 or 40 x 10^7 spermatozoa or cervical insemination using 80 x 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

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
Treatment Groups	GP1	GP2	GP3	GP4
Insemination dose (sperm)	10×10^7	20×10^7	40×10^7	80×10^7
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 least at $P < 0.05$

DISCUSSION

In the current study, the lambing rate after laparoscopic insemination using 20×10^7 spermatozoa (group 2) was significantly ($P \leq 0.05$) higher than that after cervical insemination using 80×10^7 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 (10×10^7) resulted in decreased lambing rate (37.50%), whereas a higher insemination dose (20×10^7) 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 (40×10^7) 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 x 10^7 spermatozoa per dose in intrauterine inseminations of ewes. On the other hand, Leethongdee (2010) recommended a minimum number of 40 x 10^6 spermatozoa per laparoscopic insemination. Also, Evans and Maxwell (1987) recommend a minimum dose of only 20 x 10^6 motile sperm while there are several reports of acceptable fertility (> 50%) using doses as low as 5 x 10^6 (Eppleston *et al.*, 1986) and 10 x 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 x 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 البرقى بالمنظار داخل الرحم (بإستخدام جرعات مختلفة من السائل المنوى) وداخل عنق الرحم

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يهدف هذا البحث إلى دراسة كفائة تلقيح النعاج البرقى داخل الرحم بالمنظار مقارنة بالتلقيح بعنق الرحم بالإضافة لمعرفة العدد الأمثل من الحيوانات المنوية للتلقيح داخل الرحم. تم لإجراء هذا البحث إستخدام ٣٣ نعجة برقى بعد عمل تزامن شبقى لها وتم تقسيمهن إلى أربع مجموعات ولقحت المجموعات الثلاثة الأولى منها داخل الرحم بالمنظار بإستخدام ١٠ X 10^7 (المجموعة الأولى) و ٢٠ X 10^7 (المجموعة الثانية) و ٤٠ X 10^7 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 فى المجموعة الأولى والثالثة والرابعة (٣٧.٥٠%، ٦٢.٥٠% ، ٥٥.٥٦% ، على الترتيب). يستنتج من هذا البحث أن حقن السائل المنوى للكباش داخل الرحم عن طريق المنظار البطنى يعتبر تقنية سهلة التطبيق تحت ظروف الحقل لتحقيق معدل مرتفع من الولادات وتوصى نتائج البحث بأفضلية إستخدام ٢٠ X 10^7 حيوان منوى كجرعة مثلى لتلقيح النعاج البرقى بإستخدام هذة التقنية.