REPRODUCTIVE EFFICIENCY OF DESERT SHEEP FOLLOWING SURGICAL INTRA-UTERINE ARTIFICIAL INSEMINATION IN THE SUDAN

Mohamed Nour, M. S., [1*] **Musa, S. M**[1]**., , S. A Makawi.**[1]

[1]Department of Reproduction and Obstetrics, Faculty of Veterinary Medicine, University of Khartoum, P. O. Box 32 Khartoum North, Sudan

المستخلص

اجريت هذه التجربة لتقييم كفاءة التلقيح الإصطناعي في الرحم جراحياً باستخدام السائل المنوي الطازج المخفف على الكفاءة التناسلية للضأن الصحراوي في السودان .
تم اختيار ثلاثين نعجة حمرية لهذه الدراسة متوسط أعمارها ٢٧ شهراً وأوزانها ٣٠ كجم وحالتها الجسدية ٣ درجات . وزعت النعاجً إلى ٣ مجموعات متساوية وذلك لإجراء التلقيح الإصطناعي في الرحم جراحياً (IUAI)، التلقيح الإصطناعي في عنق الرحم (ICAI) والتلقيح الطبيعي (NM) (مجموعة التحكم).عولجت جميع النعاج لإحداث تزامن للشبق بإستخدام هرمون البروجسترون الحامل وذلك باستخدام لبوسات مهبلية مشبعة بالبروجسترون (CIDR 0.3 g) لمدة ١٢ يوم. عند إخراج هذه اللبوسات حقنت جميع الإناث بهرمون مصل الفرس الحامل (PMSG) بجرعة مقدارها ٣٠٠ وحدة دولية في العضل.استخدم سائل منوي مخفف بلبن مجفف منزوع الدسم بجرعة تلقيح مقدارها 0.25×10^9 حيوان منوي . أُجري التلقيح الإصطناعي في الرحم (IUAI) بعد مُضي ٥٠-٥٤ ساعة من نهاية المعالجة الهرمونية، بينما تم التلقيح الإصطناعي في عنق الرحم (ICAI عند ظهور الشبق بعد نهاية المعالجة الهرمونية. وأما النعاج التي حدد لها التلقيح

* Corresponding Author

E-mail: mohnour_99@yahoo.com

الطبيعي فقد تركت مع الكبش بعد نهاية المعالجة الهرمونية.أغلب الحويصلات القابلة للتبويض في النعاج التي أُخضعت للجراحة في المبيض الأيمن (٨٠%) وأقطارها تراوحت بين ٠.٥-٠.٨ سم.
تم حساب معدل الحمل، بمعدل عدم الرجوع للحيال وتأكيدهَ بقياس هرمون البروجسترون بعد انقضاء دورة الشبق الأولى بعد التلقيح . معدل الحمل كان عالياً بصورة معنوية(P< 0.001) في النعاج التي لُقِحت في الرحم (١٠٠%) والنعاج التي لُقِحت طبيعياً (١٠٠%) مقارنة بتلك التي لقحت في عنق الرحم (٦٠%). كان هنالك فرقا معنوياً في معدل التوائم بين النعاج التي لقحت في عنق الرحم (ICAI) وتلك التي لقحت طبيعياً (٦٦.٧%، ١٦.٧ على التوالي). في حين أنَّه لم تتم ولادة ايِّ توائم في النعاج التي لقحت في الرحم (IUAI). كما أظهرت النتائج أيضاً عدم وجود ايِّ فروق معنوية في معدل المواليد ومتوسط طول فترة الحمل بين الثلاث مجموعات. خلصت هذه الدراسة إلى أن التلقيح الإصطناعي في الرحم (IUAI) كان وسيلة فعَّالة لتحقيق نسبة حمل عالية ، باعتبار معدل عدم الرجوع للشبق ، إلا أنَّ معدل الولادات الناتج كان متدنياً، وهذا يستدعي إبتكار وسيلة مناسبة أقل اجهاداً للحيوان ولا تؤثر على استمرار الحمل، مثل منظار البطن .

Abstract

The objective of the present study was to verify the competence of intra-uterine Artificial Insemination via surgical interference using fresh diluted semen in improving reproductive performance in Desert ewes compared to the conventional methods.

Thirty Hamari ewes were selected with average age of 27 months, body weight of 30 kg and body condition score of 3 . They were equally divided into 3 groups (10 ewes each). The three groups were randomly assigned to intra-uterine artificial insemination (IUAI), intra-cervical artificial insemination (ICAI) and natural mating (NM). Oestrus was synchronized and induced in all ewes using progesterone and pregnant mare serum gonadotrophin (PMSG). The ewes were fitted with controlled internal drug releaser (CIDR, 0.3 g progesterone) for 12 days. At the time of CIDR withdrawal, ewes received 300 IU of PMSG intramuscularly. Artificial insemination (IUAI & ICAI) was performed using freshly collected semen diluted in reconstituted skim powdered

milk. Intra-uterine artificial insemination was conducted 50-54 hours post PMSG treatments, while the intra-cervical insemination was conducted at heat detection after progesterone and PMSG treatment. Ewes assigned for natural mating were left with the ram just after the hormonal treatment.
Most of the ovulatory follicles in ewe's undergone surgery were found in the right ovary (80%) with a diameter ranged between 0.5 to 0.8 cm. The conception rate as detected by the non-return rate using a vasectomized ram and confirmed by progesterone assay was significantly higher ($p < 0.001$) for ewes undergone intra-uterine insemination (100%) and natural mating (100%) compared to cervical insemination (60%). In contrast, the lambing rate of the ewes undergone surgical intra-uterine insemination was significantly lower ($p < 0.001$) as compared to ewes inseminated intra-cervically or naturally mated. There was a significant difference in the twining rate for ICAI and NM (66.7% and 16.7%, respectively) whereas no twins were born by ewes undergone IUAI. There were no significant differences ($P > 0.05$) in litter size and gestation length for all types of insemination.
In conclusion, the present results indicate that the intra-uterine artificial insemination was an efficient method in ewes to achieve high conception rates; as shown by the non-return rate , despite the low lambing rate .However, a suitable less stressful methods such as laparoscopic insemination could be used in future studies.

Introduction

Although the population of sheep is significantly larger than that of cattle, they are often neglected in most development programmes in the tropics (Carels, 1983). Sudanese sheep had been classified according to their physical features and ecological distributions into five major groups (Mcleroy, 1961). The main group is the Desert sheep which constitutes over 60% of the total sheep population in the country (Medani, 1996). This group has reputable production and marketing features in comparison to other local types. Its outstanding merits include large body, high dressing percentage and excellent mutton. Many breed-type of Desert sheep exist and they are named after the tribe or locality where they are raised (i.e. Hamari is named after a tribe called Hamar).

Artificial insemination is the oldest and currently most common technique in the assisted reproduction of animals; it represents by far, the most notable success to date of applying reproductive technology in sheep industry and it is the best method of rapidly applying basic research data to a widespread industrial practice (Hunter, 1980; Verberckmoes, et al., 2004). Ewes can be artificially inseminated using different methods, such as vaginal artificial insemination (Evans and Maxwell, 1987) intra-cervical artificial insemination (Andersen and Aamdal, 1972) transcervical artificial insemination (Windsor, et al., 1994) and intra-uterine artificial insemination (Fukui et al. 2008; Hiwasa et al. 2009). Artificial insemination had not been widely used in Sudanese sheep as a routine commercial practice, despite the good encouraging results of intracervical artificial insemination (Alsayed, 1996;; Elmubark, 2001; Makawi and Manahil, 2007).

Intra-uterine artificial insemination is the most significant development in sheep artificial insemination. It can be performed either by transcervical insemination (Meghan, et al., 2002) through laparoscopy (Hill, et al., 1998) or via laparotomy (Evans and Maxwell, 1987; Meghan, et al., 2002). Intra-uterine artificial insemination is conducted to circumvent the problems of the traditional methods of artificial insemination (Killeen and Caffery, 1982; Evans, 1988), increase the use of highly selected rams in genetic improvement programmes (Chemineau, et al., 1991) ensure a high fertility rate (Verberckmoes, et al., 2004; Luther et al.,2007), improve the fertility rates after insemination with low numbers of sex-sorted spermatozoa (Verberckmoes, et al., 2004), improve the fertilization rate of oocytes in superovualted females in embryo transfer programme (Chemineau, et al., 1991), and to overcome sub-fertility and unexplained infertility of the male and immunological disorders of idiopathic sterility (Killeen and Caffery, 1982; DoAmard, et al., 2001; Verberckmoes, et al., 2004).

As the cervix in ewes is difficult to penetrate (Meghan, et al., 2002, Mitchell, et al., 2002), and intra-uterine artificial insemination through the cervix is rather difficult, surgical procedure was selected to deposit the semen inside the uterus , in this study.

Materials and Methods

Ewes

Thirty Hamari ewes of 1–2 previous lambings without obstetrical problems were selected for this study from Khartoum University Farm. The ewes were apparently clinically free from any detectable diseases. Their average age, weights, and body condition score, were 27 months, 30 kg and 3 respectively. All ewes had de-wormer drench (Vety Alben, Leads Pharma, Pvt, Ltd, Islamabad, Pakistan) and were sprayed with insecticidal drugs (Cypermethrin, Veterinary & Agricultural Products Mfg Co. Ltd, Amman, Jordan) to eliminate external parasites. They were fed on had free green fodder *ad-libitum* (alpha alpha),and 0.5 kg/head/day concentrate ration composed of (50% groundnut cakes + 50% wheat bran). The animals were given free access to clean drinking water throughout the day.

Oestrous Synchronization

Oestrous cycle in all ewes was synchronized using controlled internal drug release device (CIDR) containing 0.3 grams progesterone (EAZI – BREED CIDR, Pharmacia & Upjohn Ltd Company, Auckland). The intra-vaginal devices were introduced and remained in situ for 12 days as recommended by the CIDR manufacturer. All ewes received 300 IU PMSG (Folligon, Intervet, Cambridge, UK Ltd) intramuscularly, at the time of CIDR withdrawal.

Semen Collection and Processing

Semen from a Hamari ram, of a proven history of excellent fertility, was collected using an artificial vagina. Briefly, the ejaculates were evaluated microscopically for volume, motility (wave motility, individual motility, percentage of motile spermatozoa), concentration, live/dead ratio and morphology of individual spermatozoa. The ejaculates were diluted in a skim powdered milk extender to give an insemination dose of $0.5X10^9$ spermatozoa/ml packed in mini-straws of 0.5 ml (Minitub-Abfül-und, Labortechnik GmbH & Co. K G, Germany).

Artificial Insemination

After oestrous synchronization, the ewes were equally divided into three groups, considering the age , body weight and body condition score . Each group was randomly assigned to one of the following insemination procedures:

Intra-cervical Artificial Insemination (ICAI)

Intra-cervical artificial insemination was followed as reported by Chemineau et al. (1991), briefly, the insemination gun, loaded by a semen straw, was introduced under the small tongue of the cervix by pushing the gun carefully with rotary motion as far as the cervix allowed its passage (Fig. 1). Semen was pushed by the plunger into the cervix.

Intra-uterine Artificial Insemination (IUAI)

Surgical IUAI was conducted as described previously by Musa, (2004). As illustrated in Fig. 2 (a, b, c, and d), ewes were prepared aseptically, the abdominal cavity was opened on the side of one udder obliquely from the medio-caudal to the latero-cranial aspect (Fig. 2 a and c). The uterus was then exposed through the incision. The diluted semen (0.5 ml containing $2.5X10^8$ spermatozoa) was transferred into the uterus by using one ml syringe (26GX½", Kana Corporation, Korea). The needle of the syringe was stabbed through the uterine wall into the lumen approximately halfway between the uterine bifurcation and the utero-tubal junction and pushed to expel the semen in the uterine lumen (Fig. 2. d).

Pregnancy diagnosis

All ewes were monitored for return to oestrus by the aid of a vasectomized teaser ram until day 17 post insemination. Pregnancy rate was considered as the percentage of ewes not returning to heat . Progesterone assay (Pathozyme progesterone, Omega Diagnostic Ltd, Scotland, UK) was used to confirm the pregnancy at day 17 post insemination .

Statistical analysis

Reproductive parameters were analyzed by chi-square with Yat`s correction using Stat view software (A basic concepts Inc., Berkely, CA, USA). A probability of less than 0.05 was considered significant. The lambing rate, twining rate, litter size and gestation length were also calculated.

Results

Table (1) shows the effect of different insemination methods on the reproductive parameters of the ewes. The conception rates as detected by the non-return rate and confirmed by progesterone assay for intra-uterine insemination, cervical insemination and natural mating, are shown in table (1) and the progesterone concentration on day 0 and day 17 is shown in table (2). The conception rate was significantly higher ($p<0.001$) for ewes undergone intra-uterine insemination (100%) and natural mating (100%) compared to cervical insemination (60%0) as shown in table (1).

The lambing rate of the ewes received intra-uterine insemination was significantly lower ($p<0.001$) as compared to those received cervical insemination or undergone natural mating (Table, 1). Twining rate was significantly higher for ewes with intra-cervical insemination than those undergone natural mating. No twins were born to those received surgical intra-uterine insemination (Table, 1). Litter size was the same for all types of insemination ($p> 0.05$) as in table (1). No significant difference (p> 0.05) in the gestation length was noticed for the three methods of insemination (Table, 1).

The diameter of graffian follicles in both ovaries for ewes assigned to intra-uterine artificial insemination after surgical opening is shown in table (3). The diameter of follicles ranged between 0.5-0.8 cm (Table 3). The majority of the follicles (80%) were observed in the right ovary.

Table 1 Effect of different insemination methods on reproductive parameters of the experimental ewes

Investigated Parameter	Insemination Method		
	IUAI	ICAI	NM
Pregnancy at day 17 post insemination			
Non-return Rate (%)	100^{b}	60.0^{a}	100.0^{b}
Progesterone Assay (%)*	100^{b}	60.0^{a}	100.0^{b}
Lambing Rate (%)	20.0^{b}	60.0^{a}	66.60^{a}
Twining Rate (%)	0.00^{b}	66.7^{a}	16.70^{c}
Litter Size	1.0	1.6	1.2
Gestation Length (days)	153 ± 0.7	153 ± 2.9	154 ± 2.9

^{a,b,c} Values with different superscript within raw differ significantly (p at least < 0.05)

IUAI = Intra-uterine Artificial Insemination

ICAI = Intra-cervical Artificial Insemination

NM = Natural mating

* Ewes with progesterone level above 3.7 ng/ml

Table 2 Progesterone concentration at day 0 and day 17 post insemination for intra-uterine, intra-cervical insemination and natural mating

Insemination Method	Progesterone Concentration (ng/ml	
	Day zero	Day 17
IUAI*	1.1 ± 0.8	9.9 ± 7.4
ICAI*	1.3 ± 0.7	8.6 ± 9.9
NM **	1.1 ± 0.7	7.0 ± 4.1

* Values are mean ± SD of 10 replicates

** Values are mean ± SD of 9 replicates

IUAI = Intra-uterine artificial insemination

ICAI = Intra-cervical artificial insemination

NM = Natural mating

Table 3 Diameter and number of ovulatory follicle for ewes undergone surgical intr-uterine insemination

Follicle Diameter (cm)*	0.7 ± 0.1
Number of Ovulatory follicle in the right Ovary **	11
Number of Ovulatory follicle in the left Ovary **	2

Values are mean ± SD of 9 replicates

** Values are the number of follicles obtained from 9 ewes

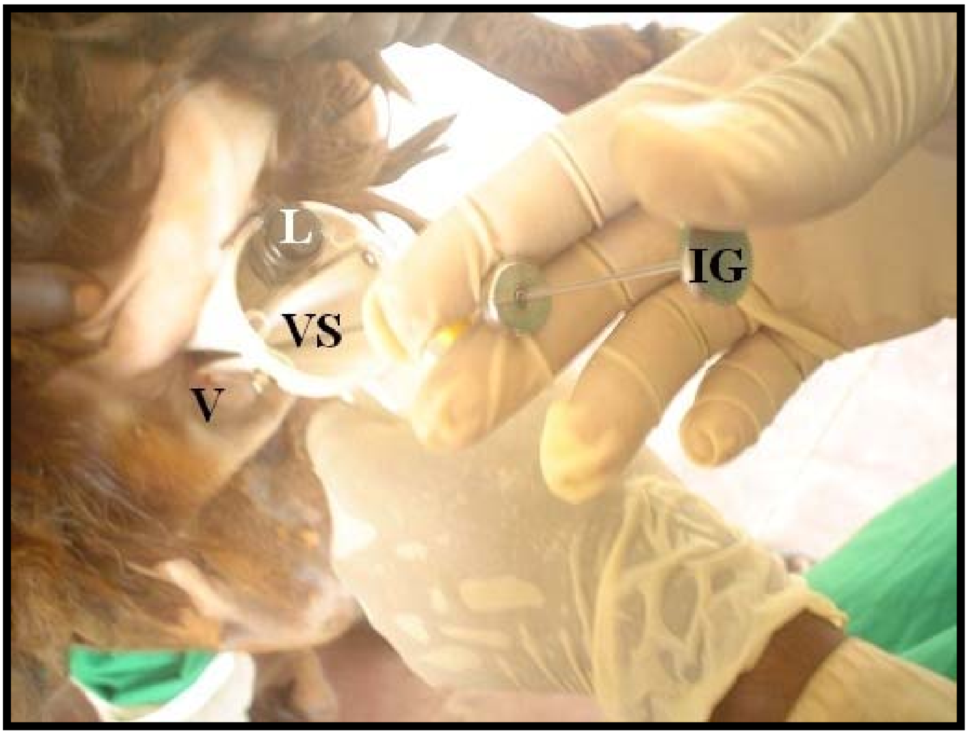

Fig.1. Intra-cervical Artificial Insemination

V = vagina
VS = Vaginoscope
L = Lamp
IG = Insemination gun

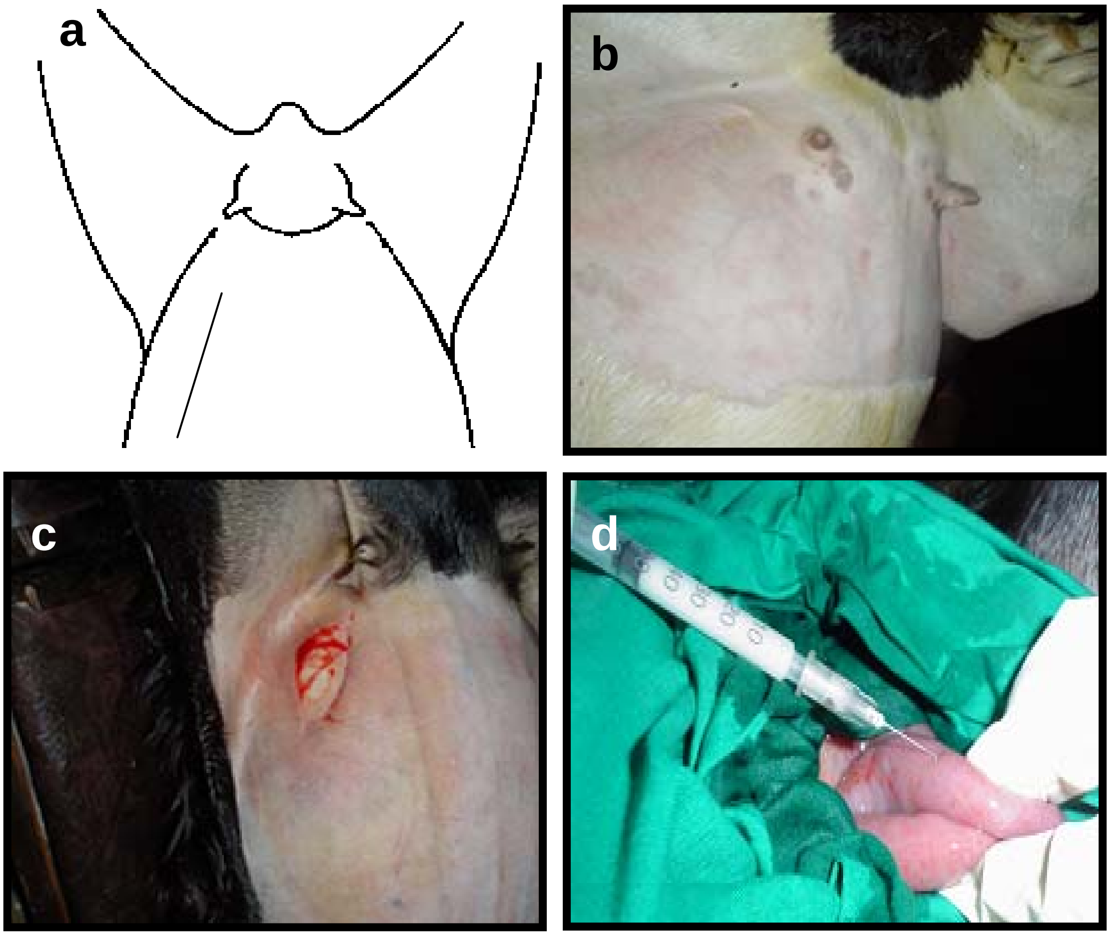

Fig.2. Intra-uterine Artificial Insemination a) site of the surgical incision b) site preparation, c) Surgical incision, and d) sperm injection in the uterine horn

Discussion

In this study pregnancy rate was significantly higher (100 %) in intra-uterine artificial insemination and natural mating, as confirmed by plasma progesterone analysis. This high pregnancy rate is in agreement with previous report indicating that conception rates were increased in ewes inseminated by semen deposition into the uterine horn (Andersen, et al., 1973; Lawrenz, 1985; Warren, et al., 1989; Fukui, et al., 1993; Hill, et al., 1998; D`Alessandro, et al., 2001). The high pregnancy rate for intra-uterine artificial insemination in this study, was attributed to the fact that all ewes had follicles of diameters ranging between 0.5-0.8 cm. According to literature, these follicles were destined to ovulate (Hafez, 1975). Moreover, the appropriate time of insemination and the direct deposition of semen in the uterus are also contributing factors.

Although the intra-uterine method of insemination resulted in higher pregnancy rate, in this study, the lambing rate was significantly lower (20%) than intra-cervical artificial insemination and natural mating (60% and 66.6% respectively). Many authors reported high lambing rate after laparoscopic intra-uterine insemination ((Luther,et al., 2007; Fukui et al. 2008; Hiwasa et al. 2009;). However, low percentage of lambing obtained in this study accords with previous reports using the same techniue (Halbert, et al., 1990; Buckrell, et al., 1994; O`Brien and Vandekerckhove, 2000) and this result was attributed to surgical stress. Nevertheless, these results could have been better if a large number of ewes were used, or less invasive method of intrauterine artificial insemination was adopted.

No twins were born to ewes subjected to surgical intra-uterine artificial insemination. Though, high rate was obtained under the present experimental conditions as shown in those undergone ICAI. This could be due to the small number of lambed animals in intra-uterine inseminated group.

The gestation length in this study accords with those obtained in Desert ewes (Makawi and Manahil,2007). However the gestation length in this study was longer than those recorded in Awassi ewes (Zarkawi, et al., 1999), Dorset Horn and Southern ewes (Clegg, 1959), Menz sheep (Mukasa and Lahlou, 1995).

The present data, considering the non-return rate, showed that intra-uterine artificial insemination significantly improves the conception rate. The maintenance of pregnancy to full term under surgical condition needs to be investigated. Trial with less invasive techniques could be adopted in the future.

References

Alsayed, A. A., 1996. Reproductive characteristics and introduction of artificial insemination in desert sheep. M.V.Sc. Thesis, University of Khartoum, Khartoum.

Andersen, J., J. Aamdal, and J. A. Fougner, 1973. Intrauterine and deep cervical insemination with frozen semen in sheep. Zuchthgiere. 8:113-118.

Andersen, M. and J. Aamdal, 1972. Artificial insemination with frozen semen in sheep in Norway. World Review of Animal Production. 8:77-79.

Buckrell, B. C., C. Bushbeck, C. J. Gartley, T. Kroetsch, W. McCutcheon, J. Martin, W. K. Penner, and J. S. Walton, 1994. Further development of a transcervical technique for artificial insemination in sheep using previously frozen semen. Theriogenology. (42):601-611.

Carels, A. B., 1983. Sheep production in the tropics. Oxford University Press, London.

Chemineau, P., Y. Cagnié, Y. Guérin, P. Orgeur, and J. C. Vallet, 1991. Training manual on artificial insemination in sheep and goats. FAO-Animal Production and Health.

Clegg, M. T., 1959. Factors affecting gestation length and parturition. Page 543 *in* Reproduction in Domestic Animals. 1st edition ed. H. N. Cole and P. T. Cupps, eds. Academic Press, New York.

D`Alessandro, A. G., G. Martemucci, M. A. Colona, and A. Bellitti, 2001. Post-thaw survival of ram spermatozoa and fertility after insemination as affected by prefreezing sperm concentration and extender composition. Theriogenology. (55):1159-1170.

DoAmard, V. F., R. A. Ferriani, R. M. Dos Reis, M. M. De Sale, and M. D. De Moura, 2001. Effect of inseminated volume on intrauterine insemination. Journal of Assisted Reproduction. 18:413-416.

Elmubark, A. A., 2001. Fertility rate after oestrous synchronization and artificial insemination in desert Sheep under extensive system in the Sudan. M.V.sc. Thesis, University of Kkartoum, Khartoum.

Evans, G., 1988. Current topic in artificial insemination of sheep. Australian Journal of Biology and Science. 41:103-116.

Evans, G. and W. M. C. Maxwell, 1987. Salamon's Artificial Insemination of Sheep and Goats. 1st edition ed, Sydney.

Fukui, Y. , Kohno, H. , Togari, T. , Hiwasa, M. and K. Okabe, 2008 . Fertility after artificial insemiation using a soyabean - based semen diluent in sheep .Journal of Reproduction Development, 54 (4) : 286 - 289 .

Fukui, Y., H. Hirai, K. Honda, and K. Hayashi, 1993. Lambing rates by fixed-time intrauterine insemination with frozen semen in seasonally anoestrous ewes treated with progesterone impregnated sponge or CIDR device. Journal of Reproduction and Development. 39:1-5.

Fukui, Y. and E. M. Roberts, 1977. Fertility of ewes treated with prostaglandin F2α and artificial insemination at predetermined intervals there after. Australian Journal of Agriculture Research. 28:891-897.

Hafez, E. S. E., 1975. Reproduction in Farm Animals 3rd edition ed. Bailliere Tindall & Cox, London.

Halbert, G. W., H. Dobson, J. S. Walton, P. Sharpe, and B. C. Buckrell, 1990. Field evaluation of a technique for transcervical intrauterine insemination of ewes. Theriogenology. 33:1231-1243.

Hill, J. R., J. A. Thompson, and N. R. Perkins. 1998. Factors affecting pregnancy rates following laparoscopic insemination of 2447 Merino ewes under commercial conditions: a survey. Theriogenology. 49:697-709.

Hiwasa, M.,Kohno, H. Togari. T. Okabe, K. and Y. Fukui, 2009 . Fertility after different artificial insemination methods using a synthetic semen extender in sheep. Journal of Reproduction Development , 55 (1) : 50 - 54 .

Hunter, R. H. F, 1980. Physiology and Technology of Reproduction in Female Domestic Animals. Academic Press INC, London.

Killeen, I. D. and G. J. Caffery, 1982. Uterine insemination of ewes with the aid of a laparoscope. Australian Veterinary Journal 46:327-333.

Lawrenz, R, 1985. Preliminary results of non-surgical intrauterine insemination of sheep with thawed-frozen semen. Journal of South Africa Veterinary Association. 56:61-63.

Luther, J. S., Grazul - Bilska, A.T., Kirsh, J.D., Weigl, R.M., Kraft, K. C., Navanukraw, C., Pant, D., Reynolds, L.P. and D. A.Redmer (2007). The effect of GnRH , eCG and Progestin type on estrous synchronization following laparoscopic AI in ewes . Small Ruminant Research , 72 : 227 - 231.

Makawi, S. A. and Z. A. Manahil , 2007 .Fertility response of Desert ewes to hormonal oestrous synchronization and artificial insemination using fresh dilutes semen. Journal of Animal and Veterinary Advances. 6 (3) : 385 - 391.

Mcleroy, G. B., 1961. The sheep of the Sudan. 2-Ecosystem and tribal breeds. The Sudan Journal Veterinary Science and Animal Husbandry. 2:101-105.

Medani, M. A. E., 1996. Livestock and Animal Production in the Sudan. Khartoum University Press, Khartoum (in Arabic).

Meghan, C., R. Wulster, and S. L. Geogory, 2002. Development of a new transcervical artificial insemination method for sheep: effects of a new transcervical artificial insemination catheter and traversing the cervix on semen quality and fertility. Theriogenology. 58:1361-1371.

Mitchell, S. E., J. J. Robinson, M. E. King, W. A. McKelvey, and L. M. Williams, 2002. Interleukin-8 in the cervix of non-pregnant ewes. Reproduction and Fertility Development. 124: 409-416.

Mukasa, M. E. and K. A. Lahlou, 1995 Reproductive performance of Menz sheep in the Ethiopian Highlands. Small Ruminant Research. 17:167-177.

Musa, S. M., 2004. Study of Surgical Intrauterine Artificial Insemination Efficiency in Sudanese Desert Sheep. M.V.M.Thesis University of Khartoum, Khartoum.

O`Brien, P. and P. Vandekerckhove, 2000. Intra-uterine insemination versus cervical insemination of donor sperm for subfertility. *in* Cochrame Database System. Vol. 2.

Verberckmoes, S., A. Van Soom, and A. de Kruif, 2004. Intrauterine insemination in farm animals and humans. Reproduction in Domestic Animals 39:195-204.

Warren, J. E. J. R., D. O. Kiesling, M. A. Akinbani, E. A. Price, and S. Meredith, 1989. Conception rates in early post partum ewes bred naturally or by intrauterine insemination. Journal of Animal Science. 67:2056-2059.

Zarkawi, M., M. R. Al-Merestani, and M. F. Wa`rdeh, 1999. Induction of synchronized oestrous and early pregnancy diagnosis in Syrian Awassi ewes, out side the breeding season. Journal of Small Ruminant Research. 33:99-102.