RESULT OF ADVANCED TECHNIQUES FOR ARTIFICIAL INSEMINATION IN EWES WITH FROZEN-THAWED SEMEN

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SUMMARY

In all domestic species, acceptable pregnancy rate with frozen-thawed semen is 20-30% through simply cervical and vaginal insemination. Researchers have proved that achievable high results can be expected only from intrauterine insemination in ewes. Unlike the cow, and goat, the cervix of ewes is a formidable barrier to penetrate for transcervical intrautraine insemination. It is not only due to the physical size of the canal and a narrow luminal diameter (even at estrus), but also due to its caudally facing eccentric series of four to eight funnel-like rings. The major aim of this study was to evaluate a comparison of thranscervical and laparoscopic intrautraine artificial insemination techniques with frozen –thawed semen on the reproductive performance of ewes.

58 ewes of 3-4 years of age, so called Altanbulag subspecies of breed were selected and equally divided into 2 groups for the AI methods from October 15-20, 2013 in the breeding station of Research Institute of Animal Husbandry in Hongor sum, Darhan uul province. The estrus was synchronized with EAZI-BREED ^{TM} CIDR in which the progesterone content was 0.3 g. After 12 days, the CIDR were removed, PMSG of 400 and 200 IU were administrated intramuscularly per ewe. The total success of synchronization rate was 93.3%. The results has shown the possibilities of laparoscopic and trancervical AI techniques in 55.17%, 41.37% ($p>0.05$) of pregnancy rates respectively, with fozen-thawed semen in ewes by ultrasonography diagnosis.

KEY WORDS: Estrus synchronization, laparoscopic and transcervical artificial insemination, ultrasonic pregnancy detection, ewes.

INTRODUCTION

In all domestic species, acceptable pregnancy rates with frozen-thawed semen are only achievable with intrauterine insemination. Laparoscopic artificial insemination technique (LAI) was developed by Killen and Caffery (1982) and Halbert et. al. (1990) initiated the Guelph System for transcervical artificial insemination (GST-AI) to circumvent the anatomical complexities of ewes' cervix and provide acceptable pregnancy rates. In ewes, the second challenge is impractical AI due to difficulties of detecting estrus and controlling estrus cycle. The inability to freeze ram semen is also another factor that limited a wider use of artificial insemination (AI-Saadi, 2001). However, today, with the use of hormones such as progesterone, pregnant mare serum gonadotropin (PMSG) and other reproductive hormones, the synchronization of estrus in ewes are possible; in addition to that ram semen can now readily be frozen which opens the door for domestic as well as international

movement of semen. Also, laparoscope was used in artificial insemination in ewes by direct manipulation of semen into the uterine horn as means of genetic improvement (Dally, 2008).

Conception rates via transrectal or transabdominal ultrasound ranges from 32% (Windsor et al., 1994) to 64% (Smith et al., 1995), and 48% (Windsor et al., 1994) to 72% (Hill et al., 1998) for GST-AI and LAI, respectively.

Lambing rate for LAI with the frozen –thawed semen was much higher ($P<0.1$) than for GST-AI (43% vs. 20.7%, respectively) and ewes inseminated at <53 hours post –progestagen pessary removal had a lower lambing rate than ewes inseminated at >53 post-PPR (22% vs. 42.7% respectively), (B.C. Mckusick, D. L. Thomas et al., 2012).

Poff, Gerard John et al., (1996) reported that the percentage of ewes conceived to transcervically inseminated with fresh semen (42%) was greater than that for frozen-thawed (24%) ($P<0.05$). Relatively very few author's studies were reported in comparison of the two AI methods with frozen –thawed semen in ewes.

Therefore, the aims of this study were to evaluate the effects of TC-AI and LAI on the conception with frozen-thawed semen and to detect pregnancy rate at the early age of fetus by ultrasound in ewes.

MATERIALS AND METHODS

The study was conducted during the breeding season. 58 ewes, so called Altanbulag subspecies of breed were selected and divided equally into 2 groups for the AI methods with frozen- thawed ram semen either by transcervical insemination or LAI once on October 15-20, 2013 at the breeding station of Research Institute of Animal Husbandry in Hongor sum, Darhan uul province. The ewes were at the ages of 3-4 years. All animals were fed with only pasture and water was available every day.

The estruses were synchronized with CIDR (EAZI-BREED ^{TM}, 0.3 g progesterone) for 12 days, after the CIDR removal, PMSG of 400 and 200 IU were administrated intramuscularly per ewe.

Detection of oestrus was performed 24 h after injection (Day 12). 93% of ewes showed oestrus within 48 h of CIDR withdrawal.

Semen was frozen in 0.25 ml straws, each containing approximately 117 million live sperms. During each of experiment, the straws were thawed and evaluated by the percentage live motile sperm on the Sperm Automatic Inspection Analysis System. The average percentage of motility rate of semen was 26±1.1%. All the ewes were fasted for 24 hours prior to inseminations.

Ewes for LAI were restrained in dorsal recumbence in a 30 degree inclined laparotomy cradle. 1 ml of 2% lidocaine hydrocholoride (Phoenix Pharmaceutical, Inc., St. Josep, MO) was administered subcutaneously at two paramedian sites. 10 cm cranial to the udder and 2 cm at either side of the ventral midline. One centimeter incisions through the skin were made over the sites of local anesthesia with a no. 10 scalpel blade. A7 mm laparoscopic trocar in a trocar sleeve, directed caudally, was subcutaneously introduced for 2 cm in the left paramedian incision and was penetrated into the abdomen. The trocar was removed. Through the right paramedian incision, a 5 mm trocar in a trocar sleeve was inserted intrabdominally with the previous technique. After locating the uterus with the laparoscope, the insemination gun (IMV, L' Aigle, France) was substituted for the 5 mm trocar and inserted intrabdominally through the right trocar sleeve. Each utrine horn was intraluminally inseminated approximately 4 cm cranial to the cornual bifurcation with one-half of the 0.25 ml straw. The instruments were removed from the abdomen and the skin incision closed with meltable surgical cords. Surgical instruments were soaked in a cold-sterilization tray with a chlorhexidine diacetate solution between ewes.

Ewes for TC –AI were restrained in dorsal recumbence. The ewes were injected for muscle attenuator (Nosh-va 2ml). Spermicidal lubricated vaginal speculum with light source was inserted intravaginally and the cervical os located by an experienced transcervical inseminator. The largest of the cervical flaps

was grasped with Bozeman forceps and retracted caudally and laterally as the speculum was advanced cranially to elongate the cervical canal. Insemination tip was passed transcervically to achieve intrauterine semen deposition. Ovary follicles during oestrus period and pregnancy diagnosis were performed by the ultrasound with transrectal riod and transabdominal probe.

RESULTS

The ovarian response or the mean±SD of the size of preovulatory follicles was 4.3± 0.12 mm at 24±075 h after the CIDR removal.

Table 1

Descriptive statistics of ultrasound detection

Variable	N	Mean	Range	Median	SD
Follicles	54	4.3	1.00; 9.3	5.0	1.72

[N]The number of preovulatory follicles (>5 mm) found at the mid oestrus in all ewes

Figure 1. Ultrasonofraphy images of the preovulatory follicle on the ovary. All anechoic structure larger than 5 mm were observed in the image.

Although impossible to determine the exact number of follicles developed after hormonal treatment of ewes, the ultrasonic exams are found to be very useful tool to estimate the tendency of follicular development and as well as to approximately predict the ovulatory rate. Also, relative deviation report can be used to predict the success of superovulation in a group of ewes, provided that ultrasound scanning was performed by the same person.

By clinical signs and use of ultrasonography, the pregnancy diagnoses were made at the first 60 days of gestation period (Figure 2). The tests of pregnancy diagnosis were performed by the use of ultrasonoraphy of the pelvic region depending on the presence of fetus or enlargement of uterine lumen.

Figure 2. Fetus inside dam uterine lumen up to 70 day of pragnacy

Table 2

Reproductive performance of ewes artifially inseminated

Item	Number of ewes present at pregnancy diagnosis	Number of pregnant ewes/ pregnancy rate (%)
LAI	29	16 (55.17)
T-AI	29	12 (41.37)
Total	58	28 (48.27)

[LAI] Laparoscopic artificial insemination, T-AI transcervical artificial insemination

Pregnacy rate for LAI was not much higher (P>0.05) than for TC-AI (55.17 vs.41.37), this difference was thus not much statistically significant.

DISCUSSION

The ultrasonic exam is a great tool to follow up the follicle growth and timely insemination before ovulation period. Our result of measurement of ovary follicles agree with the study of Juraj Grizel et al.(2013). They reported that at oestrus onset, 48 h after ovulation and at the beginning of oestrus, the size of unovulated and luteinized follicles were >5mm and >4mm respectively.

Campbell et al., (1996), and Mckelery (1994) and B.C. Mckusick et. al have reported there is epithelial damage to the lining of cervix ranging when the GST-AI technique is used. Thus, it is possible that ewes that do not achieve conception to GST-AI could have decreased fertility.

Hence, to avoid from this problem we used the injection for muscle attenuator (Nosh-va 2ml) and the special catheter used for human IUI. Windsor et. al (1994) did not find significant differences in fertility between GST-AI and LAI, which agrees with our results.

CONCLUSION

The ultrasound with transrectal probe can be used to make an accurate measurement of ovary condition, whereas transabdominal ultrasound is helpful to employ the prediction pregnancy, gestatinal age, and fetal number between 40-60 days in ewes.

Although laparoscopic insemination is superior to transcervical insemination, insemination performed after 48 hours post CIDR removal resulted in closer fertility rates in the 2 methods used.

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