

Effect of season and mating system in Awassi ewes superovulated with FSH on fertilization rate and embryo recovery

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Abstract

The aim of the present study was to evaluate the contribution of laparoscopic intrauterine insemination to the improvement of fertilization and embryo recovery in Awassi ewes superovulated with FSH in breeding and non-breeding season. Twelve nonpregnant and cycling Awassi ewes of 3-4 years of age were randomly allocated in equal numbers ($n = 6$) to two groups. Each ewe was treated with a progesterone impregnated intravaginal sponge for 12 days. All ewes were superovulated with FSH in eight reducing doses for four days in the morning and evening from two days prior to sponge withdrawal. Ewes of group 1 were mated naturally at least two times with Awassi rams of proven fertility. Ewes of group 2 had intrauterine insemination and were conducted 44-46 h after sponge removal, under laparoscopic visualization of uterine horns, depositing 1 ml of semen in the distal portion of each uterine horn. Ovarian response was assessed by determining number of corpora lutea by laparoscopy on day 6 after mating. Embryo recovery was performed by hand assisted laparoscopy and by flushing both uterine horns. Ovarian response of the ewes superovulated with FSH was decreased to half in the non-breeding season. High number of unfertilized ova ($P < 0.05$) was observed in ewes superovulated with FSH in the non-breeding season when naturally inseminated compared to ewes inseminated intrauterine using laparoscopic technique. Higher rates of embryo recovery ($P < 0.05$) were achieved in superovulated ewes in the breeding season when ewes were inseminated by laparoscopic intrauterine insemination. The fertilization rates in ewes inseminated intrauterine using laparoscopic techniques in breeding season and non-breeding season were 88.1% and 37.98%, respectively. It could be concluded from the results of the present study that the use of FSH to induce superovulation in Awassi ewes combined with laparoscopic intrauterine insemination can increase the fertilization rate in the breeding season.

Keywords: Sheep; Superovulation; FSH; Embryo recovery; Awassi breed.

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(P<0.05)

37.98% 58.05%

(P<0.05)

Introduction

Awassi sheep is a highly productive indigenous dairy breed as well as producing wool and meat. Studies concerning the superovulatory, laparoscopic intrauterine insemination and embryo recovery were scarce in Awassi ewes and to our knowledge, no studies have been reported in Iraq developing a protocol that could be easily applied in extensively managed flocks for embryo transfer. Superovulation is prerequisite for the collection of larger than normal number of embryos and for the realization of a commercially applied embryo transfer programs. Recent research (1-3) efforts are directed at improving the superovulation efficiency and the shedding of high quality oocytes. Follicle stimulating hormone (FSH) is widely used to induce superovulation in sheep (2,4). Sheep breed, dose of hormone, season, mating system and embryo recovery techniques have been recognized as some major causes of variation in the superovulatory results (2,5,6). Data regarding the effect of season in superovulated ewes are limited (7). Estrous synchronization and superovulatory treatment interfere with sperm transport through the cervix and this in turn, compromises the fertilization rate and thereby, the supply of viable transferable embryos (8-11). Laparoscopic intrauterine insemination, which by passes sperm transport through the cervix, may prove to be a useful method for increasing fertilization rate (12-15). Techniques for intrauterine insemination and embryo recovery from ewes involved major surgery in the form of laprotomy and exposure of the uterus. The acceptability of this procedure is questionable due to the reduced fertility because of postoperative adhesions to the animal.

Laparoscopic techniques allow time efficient and minimally invasive intrauterine insemination of sheep (8,16). Killen and Caffery (17) were the first to describe the use of laparoscopy for intrauterine insemination of sheep. The advantage of laparoscopic insemination is that the semen is deposited closer to the site of fertilization (2,18).

The aim of the present study was to evaluate the contribution of laparoscopic insemination to the improvement of fertilization and embryo recovery in Awassi ewes superovulated with FSH in breeding and non-breeding season.

Materials and methods

The experiment was conducted in breeding season when major breeding activities (September 2007) commence and winter (January 2008) during complete anestrous season at the farm of the College of Veterinary Medicine, University of Mosul. Twelve nonpregnant and cycling Awassi ewes of 3-4 years of age were randomly allocated in equal number (n = 6) to two groups. None of the ewes included in this study had been previously subjected to hormonal treatments. Throughout the experimental period, the animals were kept in open front barrens were fed concentrated mixture 1kg/ewe/day and were given water ad libitum.

Estrous synchronization

Each ewe was treated with a progesterone impregnated intravaginal sponge (Synncropart 40 mg sheep sponge, Ceva Sante Animal, France) for 12 days.

Superovulation treatment

The following superovulation treatment was used; Ewes received FSH (Gonal-F75, Serono, Italy) in eight reducing doses for four days in the morning and evening from two days prior to sponge withdrawal. The doses of FSH were as the followings: first day 350 IU, second day 250 IU, third day 200 IU ending with fourth day 150 IU.

Estrous detection

Estrous in ewes were detected with the aid of aproned ram (ram: ewe = 1:12) of high sexual vigor at 6 h intervals. Ewes (group 1) standing to be mounted by the aproned ram were recorded as in estrus and mated at least two times with Awassi rams of proven fertility.

Laparoscopic intrauterine insemination

Food and water were restricted for 24 h before laparoscopy. Semen was collected with an artificial vagina from two Awassi rams of proven fertility. Semen was collected within 1 h of insemination, assessed for motility and concentration and diluted with phosphate buffer saline (PH adjusted to 6.8-7.2 with osmotic pressure 270-310 mOs), so that each 1 ml of diluted semen contained 100 x 10⁶ motile sperm. Ewes (group 2) were initially sedated

with xylazine 0.05 mg/Kg BW intravenously. Intrauterine insemination was conducted 44-46 h after sponge removal, under laparoscopic visualization of uterine horns, depositing 1 ml of semen in the distal portion of each uterine horn. Immediately following laparoscopic intrauterine insemination, the cannulas and CO₂ were removed and incisions were sutured.

Superovulatory response and embryo recovery

Ovarian response was assessed by determining number of corpora lutea by laparoscopy on day 6 after mating. Embryo recovery was performed by hand assisted laparoscopy and by flushing both uterine horns. Food was withheld 24 h prior to surgery. All animals underwent sedation using xylazine 0.22 mg/Kg BW intravenously. A local anesthesia at trocher and cannula entry sites was achieved by subcutaneous injection of 10 ml 2% lidocaine. The animals fixed on a movable surgical table in an upside-down position and underwent laparoscopy followed by shaving and disinfection of the abdomen. The abdomen was inflated with CO₂ and laparoscopic cannula and laparoscope were placed into the abdomen (ports of cannula insertion one at the umbilicus using No. 10 cannula for insertion the telescopes while 2 ports posterior to the first one by about 10 cm using cannula No. 5 for insertion of the laparoscopic instruments). Both ovaries were examined and the number of corpora lutea either normal (> 3mm) and anomalous (≤ 3mm) and large unovulated follicles (> 4mm) were recorded. Ewes showing more than three corpora lutea were considered as superovulated.

Embryo recovery was recorded as described by Bari et al. (19). Briefly, each uterine horn was flushed by insertion of a needle, attached to a sterile syringe with flushing media (modified Dulbecco's phosphate buffered saline plus 1% bovine serum and the PH adjusted to 7.2-7.6 with osmotic pressure 270-310 mOs) near the utero-tubal junction. Each uterine horn was flushed with 30 ml flushing media, collected in Petri dishes through a Foley catheter inserted in the base of the uterine horns for recovery of embryos. The collected flushing media was examined for the presence of oocytes and embryos under a stereo microscope.

Statistical analysis

The student t-test was used to evaluate the differences in superovulation response, ovulation rate and recovery rate between groups using the software Sigma stat (Jandel scientific software, V 3.1).

Results

The procedure of the laparoscopic intrauterine insemination from the first incision to closure of skin wounds lasted approximately 5 minutes. During laparoscopic intrauterine insemination and embryo recovery

no complications resulted from laparoscopic technique such as bleeding, blood clotting, postoperative adhesions were noticed in animals undergo laparoscopy.

Table 1 presents the superovulation response of ewes treated with FSH through number of corpora lutea, number of unfertilized ova and recovered embryos in breeding season and non-breeding season in two groups of ewes including naturally mated ewes and laparoscopic intrauterine insemination. Ovarian response of the Awassi ewes superovulated with FSH was decreased to half in the non-breeding season by the estimation the number of corpora lutea in ovaries. High number of unfertilized ova ($P < 0.05$) was observed in ewes superovulated with FSH in breeding season and non-breeding season when naturally inseminated compared to ewes inseminated intrauterine using laparoscopic technique. Higher rates of embryo recovery ($P < 0.05$) were achieved in superovulated ewes in the breeding season when ewes inseminated either naturally or by laparoscopic intrauterine insemination. The fertilization rates (No. of recovered embryos / No. of corpora lutea) in ewes inseminated intrauterine using laparoscopic techniques in breeding season and non-breeding season were 88.1% and 37.98%, respectively. While the fertilization rate in superovulated ewes naturally mated in breeding and non-breeding seasons were 44.89% and 24.24%, respectively. Fertilization failure (No. of unfertilized ova / No. of corpora lutea) in superovulated ewes in breeding and non-breeding seasons using intrauterine insemination were 3.96% and 9.97%, respectively. While fertilization failure in ewes superovulated in breeding and non-breeding seasons and mated naturally were 29.85% and 28.05%, respectively.

Discussion

Results of the present study demonstrated a good ovarian response could be achieved by the use of exogenous FSH for superovulation in Awassi ewes in the breeding season. These results were in agreement with Cordeiro (20). This hormone is widely used to induce superovulation in sheep (2,4,21). There is a considerable evidence in the literature that ovulation rate are stimulated in ewes in breeding season than non-breeding season (7,22). As day length increases, there is a gradual slowing of GnRH pulsatility until a pre-ovulatory LH surge can no longer be generated and estrous cyclicity ceases (6). Ovarian follicular development does not, however cease (10) and successive waves of follicles continue to develop throughout anestrus (23). These follicles can be stimulated to mature and ovulate by the administration of exogenous gonadotropins. The higher number of unfertilized ova may be attributed to failure of fertilization (24). It is well documented that estrous synchronization and superovulation interferes with sperm transport in naturally

mated ewes, which eventually result in impaired fertilization rate (6,25). For this reason, this study has improved the existing of laparoscopic intrauterine insemination techniques for application for the first time in Iraq in superovulated Awassi ewes. In this experimental study, the use of the laparoscopic intrauterine insemination technique resulted in higher fertilization rate than ewes in the second group were mated naturally. A significant higher fertilization rate following laparoscopic intrauterine

insemination was found in this study. This result is in accordance with other studies (12-15). The lower fertilization rate after natural mating is believed to have been caused by a deficiency in the intrauterine sperm migration (9,10,11,25). In the present study, the high fertilization rate in laparoscopic intrauterine insemination technique could be attributed to the fact that semen was deposited directly to the uterus avoiding the hostile environment of vagina and cervix.

Table 1. Number of corpora lutea, recovered embryos and unfertilized ova (mean ± SE) of Awassi ewes superovulated with FSH with Laparoscopic intrauterine insemination and natural mating during breeding and non-breeding seasons.

| Type of treatment | No. of corpora lutea | | No. of recovered embryos | | No. of unfertilized ova | |
|--|------------------------|------------------------|--------------------------|------------------------|-------------------------|------------------------|
| | Breeding season | Non-breeding season | Breeding season | Non-breeding season | Breeding season | Non-breeding season |
| Laparoscopic intrauterine insemination | 8.32±0.43 ^a | 4.41±0.52 ^b | 7.33±0.62 ^{ac} | 3.16±0.47 ^b | 0.33±0.11 ^c | 0.83±0.22 ^c |
| Natural mating | 8.91±0.39 ^a | 4.02±0.92 ^b | 4.00±0.52 ^{ad} | 2.16±0.82 ^b | 2.66±0.61 ^d | 2.5±0.56 ^d |

Superscripts a---b differ significantly at P< 0.05 between rows from each parameter.

Superscripts c---d differ significantly at P< 0.05 between columns from each parameter.

The success of fertilization is hampered by hormonal treatment either by estrous synchronization or by superovulation (9,10). Several months are required for ovarian follicles in the ewe to develop from primordial to preovulatory size (7). During this time, oocytes contained within the follicles gradually become competent to undergo fertilization and further development (6). The competence is only achieved around the time of the superovulatory LH surge (25). The use of exogenous FSH promotes follicle growth but oocytes contained within initially small follicles may lag behind in their development. In superovulated ewes, a number of small follicles as a proportion of the total follicles population are increased (7). Others (1,22) claimed that higher number of unfertilized ova could be due to increased in number of unovulated follicles in the non-breeding season resulted in high level of estradiol secretion causing failure of sperm transport in the female genital tract. It is likely therefore, that in the present study an increased number of corpora lutea in the breeding season was resulted from increased number of large follicles induced to ovulate. This higher results of unfertilized ova observed in this study in the non-breeding season might be due to immature oocytes at time of ovulation. Ova would have more time to complete cytoplasmic maturation following FSH treatments. Embryo recovery rate and high level of fertilization failure in ewes superovulated in the non-breeding season were lower than in those superovulated in the breeding season. Various factors affect fertilization rate in superovulated ewes such as light, temperature, nutrition, melatonin secretion, sperm transport

and type of mating system might be responsible for these differences. A poor synchrony of ovulation after sponge withdrawal in FSH treated ewes is a possible cause of fertilization failure after a programmed insemination (2). Two factors contribute to the spread in timing of ovulation, firstly, LH surges and secondly, the median time from first to last ovulation (4). A result of the present study showed that the most important factor could be contributed to fertilization rate in superovulated ewes is type of insemination technique. Higher rate of fertilization was achieved when superovulated ewes were inseminated intrauterine using laparoscopy.

It could be concluded from the results of the present study that there is an increased incidence of fertilization failure using FSH for superovulation in non-breeding season in Iraqi Awassi ewes. Laparoscopic intrauterine inseminations increase the fertilization rate in Awassi ewes superovulated by FSH in the breeding season. The use of FSH to induce superovulation in Awassi ewes combined with laparoscopic intrauterine insemination can increase the fertilization rate if used in the breeding season. This study improve an efficiency of laparoscopy for insemination and obtaining embryos for embryo transfer programs which could contribute to genetic improvement and increase the breed population size of Awassi sheep in Iraq.

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