Laparoscopic intrauterine insemination of Awassi ewes superovulated with equine chorionic gonadotropin.

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Abstract

The present study was undertaken to investigate the contribution of laparoscopic insemination to the improvement of fertilization and embryo recovery. The experiment was conducted in breeding season. Twelve nonpregnant and cycling Awassi ewes of 3-4 years of age were randomly allocated in equal number (n = 6) to two groups. Each ewe was treated with progesterone impregnated intravaginal sponge for 12 days. All ewes were superovulated with eCG (equine chorionic gonadotropin) 1200 IU once by intramuscular injection 48 h prior to sponge removal. 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 semi-laparoscopic and by flushing of both uterine horns. Results of the present study revealed high number of unfertilized ova (P<0.05) was observed in ewes when naturally inseminated than ewes inseminated intrauterine using laparoscopic technique. Higher rate of embryo recovery (P<0.05) was achieved when ewes inseminated via intrauterine (4.66 ± 0.66) than ewes naturally mated (2.16 ± 0.74). The fertilization rate in ewes inseminated intrauterine using laparoscopic techniques and naturally mated were 79.93% and 40.07%, respectively. Fertilization failure in ewes inseminated intrauterine using laparoscopic techniques and naturally mated were 7.37% and 49.35%, respectively. It could be concluded that the use of eCG to induce superovulation in Awassi ewes combined with laparoscopic intrauterine insemination can increase the fertilization rate.

Introduction

Iraqi sheep of the Awassi breed is a highly productive dairy breed as well as producing wool and meat. Improvement of the genetic potential of Awassi sheep may be produced through application of embryo transfer. 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 (1,2,3,4). Laparoscopic intrauterine insemination, which bypasses sperm transport through the cervix, may prove to be a useful method for increasing fertilization rate (5,6,7,8). Techniques for intrauterine insemination and embryo recovery from ewes involved major surgery in the form of laparotomy and exposure of the uterus. The acceptability of this procedure has been 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 (1,9,10). Killeen and Caffery (11) 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 (12,13). The aim of the present study was to evaluate the contribution of laparoscopic insemination to the improvement of fertilization and embryo recovery.

Materials and methods

The experiment was conducted in breeding season when major breeding activities (September 2007) commences at the college of veterinary medicine, university of Mosul. Twelve nonpregnant and cycling Awassi ewes of 3-4 years of age were superovulated with eCG (equine chorionic gonadotropin) 1200 IU once by intramuscular injection 48 h prior to sponge removal. 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 semi-laparoscopic and by flushing of both uterine horns. Results of the present study revealed high number of unfertilized ova (P<0.05) was observed in ewes when naturally inseminated than ewes inseminated intrauterine using laparoscopic technique. Higher rate of embryo recovery (P<0.05) was achieved when ewes inseminated via intrauterine (4.66 ± 0.66) than ewes naturally mated (2.16 ± 0.74). The fertilization rate in ewes inseminated intrauterine using laparoscopic techniques and naturally mated were 79.93% and 40.07%, respectively. Fertilization failure in ewes inseminated intrauterine using laparoscopic techniques and naturally mated were 7.37% and 49.35%, respectively. It could be concluded that the use of eCG to induce superovulation in Awassi ewes combined with laparoscopic intrauterine insemination can increase the fertilization rate.

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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 1200 IU of eCG (Synncropart) once as an intramuscular injection 48 h prior to sponge withdrawal.

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 10^6 motile sperm. Ewes were initially sedated with xylazine 0.22 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 with sutures of incisions.

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 semi-laparoscopic 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. An additional cannula was also inserted for 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. (14). 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 resulting 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 eCG through number of corpora lutea, number of unfertilized ova and recovered embryos in two groups of ewes including naturally mated ewes and laparoscopic intrauterine insemination. High number of unfertilized ova (P<0.05) was observed in ewes when naturally inseminated than ewes inseminated intrauterine using laparoscopic technique. Higher rate of embryo recovery (P<0.05) was achieved when ewes inseminated via intrauterine (0.66±4.66) than ewes naturally mated (0.74±2.16). The fertilization rate (No. of recovered embryos / No. of corpora lutea) in ewes inseminated intrauterine using laparoscopic techniques and naturally mated were 79.93% and 40.07%, respectively. Fertilization failure (No. of unfertilized ova / No. of corpora lutea) in ewes inseminated intrauterine using laparoscopic techniques and naturally mated were 7.37% and 49.35%, respectively.

Table(1):Numbers of corpora lutea, recovered embryos and unfertilized ova (mean ±SE) of Awassi ewes superovulated with eCG during breeding season mated by Laparoscopic intrauterine insemination and natural mating.

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>No. of corpora lutea</th>
<th>No. of recovered embryos</th>
<th>No. of unfertilized ova</th>
<th>Fertilization rate</th>
<th>Fertilization failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laparoscopic intrauterine insemination</td>
<td>5.83±0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.66±0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.93%</td>
<td>7.37%</td>
</tr>
<tr>
<td>Natural mating</td>
<td>5.39±0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.16±0.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.66±0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.07%</td>
<td>49.35%</td>
</tr>
</tbody>
</table>

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

**Discussion**

Results of the present study demonstrated a good ovarian response could be achieved by the use of exogenous eCG for superovulation in Awassi ewes. These results were in agreement with Mufi et al. (15). This hormone is widely used to induce superovulation in sheep (13,16,17). The success of fertilization is hampered by hormonal treatment either by estrous synchronization or by superovulation (2,14). It is well documented that estrous synchronization and superovulation interferes with sperm transport in naturally mated ewes, which eventually result in impaired fertilization rate (18). 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 (5,6,7,8). The lower fertilization rate after natural mating is believed to have been caused by a deficiency in the intrauterine sperm migration (2,14,4). 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. It could be concluded from the results of the present study that the use of eCG to induce superovulation in Awassi ewes combined with laparoscopic intrauterine insemination can increase the
fertilization rate. 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.

Acknowledgement

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tالتميمية في الرحم باستعمال الجراحة الناظورية للنوع العواسي المعاملة بفرط
الاباضة بهرمون محفز القد المكسيمالي الخيلي

أعمال إبراهيم عزاوي
كلية الطب البيطري/ جامعة الموصل

الخلاصة
هدفت الدراسة الحالية لتقديم كفاءة التتميمية باستخدام الجراحة الناظورية لزيادة نسبة الإخصاب واستخلاص الأجنة. 

أجريت الدراسة خلال الموسم التناسلي (أيار 2007). استخدمت 12 نعجة عواس ليتم توزيعها عشوائيا على مجموعتين
(6 لكل مجموعة). وتم توقيع النعج في heartbreaking باستخدام الأسفنجة المهبلية المشعة بهرمون البروجسترون لمدة 12
يوم. حققت النعجة 1200 وحدة دولية من هرمون محفز القد المشيمي الخيلي بالإضافة إلى 48 ساعة من سحب
الأسفنجات لإحداث فرط الإخصاب. تم تتميم النعجة المجموعة الأولى طبيعا بكلا عاملة وفوقة الخصوبة. أما النعجة المجموعة
الثانية فقد تم تتميمها باستخدام الجراحة الناظورية في قري الرحم. تم حساب الأستجابة المبيضة وذلك بحساب عدد
الأجسام الصفراية باستعمال الأداة المنظورية. واستخلصت الأجنة باستعمال طريقة شبه الجراحة المنظورية وذلك
باستعمال عسن لكر قري النعج. وتم قياس النتائج ارتقا مع وعويا (P<0.05) في عدد الوبيعات غير المخصصة عند
المقارنة بالنوع العواسية للهجمة المنظورية. ووجد فرق معنوي (P<0.05) على النعجة التي تم تتميمتها بالجراحة الناظورية (6.66±4.66) عن النعجة المحققة طبيعا (P<0.05). ووجد أن نسبة الإخصاب
في النعجة المحققة للجراحة المنظورية المحققة طبيعا كانت 79.3% و 40.07% على التوالي. بينما لوحظت نسبة
فوت الإخصاب في النعجة المحققة للجراحة المنظورية والمحققة طبيعا كانت 7.37% و 4.93% على التوالي.

يعتبر من الدراسة الحالية أن استعمال هرمون محفز القد المشيمي الخيلي لإحداث فرط الإخصاب مع الجراحة المنظورية
يمكن أن يزيد من نسب الإخصاب في النعجة العواسي.