A study on the effect of GnRH administration on the ovarian response of Awassi ewes treated with eCG to induce superovulation

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Abstract

The present study was undertaken to investigate the effect of GnRH administration on the superovulatory response of ewes treated with eCG in breeding season and non-breeding season at the college of veterinary medicine, university of Mosul farm. 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 a progesterone impregnated intravaginal sponge for 12 days. The following superovulation treatment was used; Ewes of group 1 received 1200 IU of eCG once as an intramuscular injection 48 h prior to sponge withdrawal. Ewes of group 2 were also received 1200 IU of eCG once as an intramuscular injection 48 h prior to sponge withdrawal and after 24 h of sponge removal ewes were injected with 80 µg of GnRH. Ewes standing to be mounted were recorded as in estrus and mated at least two times with Awassi rams of proven fertility. 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. Results of the present study showed when ewes treated in breeding season the number of corpora lutea was significantly higher ($P<0.05$) in ewes treated with eCG plus GnRH than eCG alone as 7.33±0.54 and 4.33±0.39, respectively. There was no significant difference in the number of corpora lutea in non-breeding season when ewes treated with eCG and eCG plus GnRH. High number ($P<0.05$) of unovulated follicles was observed in ewes treated with eCG in breeding season and non-breeding season as 2.45±0.25 and 1.16±0.3, respectively. Number of recovered embryos from ewes treated with eCG plus GnRH and eCG differ significantly ($P<0.05$) as 4.32±0.56 and 2.66±0.66, respectively in the breeding season. While no significant difference was observed when these hormones used for superovulation in the non-breeding season. It could be concluded that administration of GnRH 24 h after sponge removal increased ovulation rate of Awassi ewes treated with eCG for superovulation in the breeding season.

دراسة تأثير حقن هرمون محرر محفزات القند على الاستجابة المبيضية للنعاج العواسي المعاملة بفرط الاباضة بهرمون محفز القند المشيمي الخيلي

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الخلاصة

هدفت الدراسة لمعرفة تأثير حقن هرمون محرر محفزات القند للاستجابة المبيضية للنعاج العواسي المعاملة بفرط الاباضة بهرمون محفز القند المشيمي ألخيلي خلال الموسم التناسلي وخارج الموسم التناسلي في حقل كلية

الطب البيطري، جامعة الموصل. استخدمت 12 نعجة عواسي تم توزيعها عشوائيا إلى مجموعتين كل مجموعة تضمنت 6 نعجة وتم توحد الشبق في جميع النعاج باستعمال الاسفنجات المهبلية المشبعة بهرمون البروجسترون ولمدة 12 يوم. حقنت النعاج 1200 وحدة دولية من هرمون محفز القند المشيمي ألخيلي eCG بالعضلة قبل 48 ساعة من سحب الاسفنجات لإحداث فرط الاباضة. نعاج المجموعة الأولى (6 نعاج) تم حقنها 80 µg من هرمون محرر محفزات القند GnRH بعد 24 ساعة من سحب الاسفنجات. بينما نعاج المجموعة الثانية لم يتم حقنها بأي هرمون بعد إحداث فرط الاباضة. تم تسفيد النعاج طبيعيا مرتان أو أكثر بكباش معروفة خصوبتها. تم حساب الاستجابة المبيضية في اليوم السادس من بداية طور الشبق وذلك بحساب عدد الأجسام الصفراء باستعمال الجراحة المنظارية. واستخلصت الأجنة باستعمال طريقة شبة الجراحة المنظارية وذلك باستعمال غسول لكلا قرني الرحم. بينت نتائج الدراسة ارتفاعا معنويا ($P<0.05$) في عدد الأجسام الصفراء المعاملة بهرموني محفز القند المشيمي ألخيلي ومحرر محفزات القند عن المجموعة التي حقنت بهرمون محفز القند المشيمي ألخيلي لوحدة حيث بلغ المعدل 0.54±7.33 و0.39±4.33، على التوالي عند معاملة النعاج داخل الموسم التناسلي. ولم يلاحظ فرق معنوي في عدد الأجسام الصفراء خارج الموسم التناسي في كلا المجوعتين. ولوحظ إعداد كبيرة من الجريبات غير المنفلقة في النعاج المعاملة بهرمون محفز القند المشيمي ألخيلي لوحدة خلال الموسم التناسلي وخارج الموسم التناسلي 0.25±2.45 و0.3±1.16، على التوالي. ووجد فرق معنوي ($P<0.05$) في إعداد الأجنة المستخلصة من النعاج التي تمت تحفيزها بهرموني محفز القند المشيمي ألخيلي ومحرر محفزات القند عن حقن هرمون محفز القند المشيمي ألخيلي لوحدة 0.56±4.32 و0.66±2.66، على التوالي. ولم يلاحظ فرق معنوي في إعداد الأجنة المستخلصة من النعاج التي تمت تحفيزها بهرموني محفز القند المشيمي ألخيلي ومحرر محفزات القند عن حقن هرمون محفز القند المشيمي ألخيلي لوحدة خارج الموسم التناسلي. يستنتج من الدراسة الحالية أن استعمال هرمون محرر محفزات القند بعد 24 ساعة من سحب الاسفنجات المهبلية واستعمال هرمون محفز القند المشيمي ألخيلي لإحداث فرط الاباضة يمكن أن يزيد من نسبة الاباضة للنعاج العواسي خلال الموسم التناسلي.

Introduction

Sheep in semi-arid regions of the subtropical countries like Iraq are subjected to prevailing ambient temperatures and scarcity in the availability of feed and water. These stresses limit the reproductive performance of animals. Improvement of genetic potential of Iraqi sheep may be produced through application of multiple ovulation and embryo transfer. Improvement in the genetic potential of Awassi sheep may be produced through application of embryo transfer (1). Superovulation is prerequisite for the collection of large than normal number of embryos and for the realization of a commercially applied embryo transfer programs. Recent research (2, 3, 4) efforts are directed at improving the superovulation efficiency and the shedding of high quality oocytes.

Although much work has been done in this field, the wide variation in the superovulatory response is still the weakest link in the chain of events required for successful embryo transfer programs (5). Methods of superovulation have been improved by using regimens, which combine both eCG (equine chorionic gonadotrophin) and GnRH (Gonadotrophin releasing hormone) (6) to reduce the incidence of anovulatory and cystic follicles and to increase the proportion of developing follicles that ovulate. Increased ovulation and fertilization rates have been reported following the administration of GnRH in conjunction with eCG to

superovulated ewes (7, 8, 9). Furthermore, the addition of GnRH to eCG superovulatory regimens has been used to control time of ovulation in superovulated ewes (10).

The present study was undertaken to investigate the effect of GnRH administration on the superovulatory response of ewes treated with eCG.

Materials and Methods

The experiment was conducted in breeding season when major breeding activities (September 2007) commences and winter (January 2008) during complete anestrous season at the college of veterinary medicine, university of Mosul farm. 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 of group 1 received 1200 IU of eCG (Synncropart) once as an intramuscular injection 48 h prior to sponge withdrawal. Ewes of group 2 were also received 1200 IU of eCG once as an intramuscular injection 48 h prior to sponge withdrawal and after 24 h sponge removal ewes were injected with 80 µg of GnRH (Receptal, Intervet, Holland).

- **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 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.

- **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 Samartzi et al. (11). 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 (Sigma stat 2004, Jandel Scientific Software V2.0, Richmond, CA, USA).

Results

Table (1) presents the superovulation response of ewes treated with eCG and eCG plus GnRH through number of corpora lutea and recovered embryos in breeding season and non-breeding season. The number of corpora lutea was significantly higher ($P<0.05$) in ewes treated with eCG plus GnRH than eCG alone as 7.33±0.54 and 4.33±0.39, respectively. However, there was no significant difference in the number of corpora lutea in non breeding season when ewes treated with eCG and eCG plus GnRH. High number ($P<0.05$) of unovulated follicles was observed in ewes treated with eCG in breeding season and non-breeding season as 2.45±0.25and 1.16±0.3, respectively. Number of recovered embryos from ewes treated with eCG plus GnRH and eCG differ significantly ($P<0.05$) as 4.32±0.56 and 2.66±0.66, respectively in the breeding season. While no significant difference was observed when these hormones used for superovulation in the non-breeding season. High number ($P<0.05$) of unfertilized oocytes was observed in the superovulated group using eCG in both breeding season and non-breeding season.

Discussion

Results of the present study indicated that ewes undergoes superovulation using eCG plus GnRH had improved the superovulatory response by estimating the number of corpora lutea which was higher ($P<0.05$) than ewes superovulated with eCG alone. The observed difference in the superovulatory response was attributed to GnRH administration. Superovulated ewes with eCG followed by GnRH injection 24 h after sponge removal may result in increased ovulation rate. Gonzalez-Bulnes et al. (7) obtained similar results. The administration of GnRH at time early after sponge removal affects follicular development and maturation preceded by an endogenous LH surge (8). In comparison, the LH surge could be delayed in ewes superovulated with eCG and not treated with GnRH resulted in low level of corpora lutea and a high level of unovulated follicles. These results were in agreement with Samaratzi et al. (11) using various doses of eCG. The presence of a large growing follicle at the time of a superovulatory dosage of eCG (12) has been reported to decrease ovarian response to LH surge. Bettencourt et al. (14) have also observed an increased ovulation rate following GnRH administration. Similarly, Jabbour and Evans (9) demonstrated that the administration of GnRH 24 h after sponge removal to ewes superovulated with 1200 IU of eCG during the breeding season increased ovulation rate. In contrast, Walker et al. (14) reported no differences in ovulation rate for ewes treated with GnRH in breeding season in combination with eCG. This disagreement could be due to the difference in the dose and GnRH agonist type used in the previous and present study.

It could be concluded that administration of GnRH 24 h after sponge removal increased ovulation rate of Awassi ewes treated with eCG for superovulation in the breeding season.

Table (1) Number of corpora lutea, Unovullated follicles, recovered embryos and unfertilized ova (mean ±SE) of Awassi ewes superovulated with eCG plus GnRH and eCG during breeding season and Non- breeding season

Type of treatment	No. of corpora lutea		Unovullated follicles		No. of recovered embryos		No. of unfertilized ova	
	Breeding season	Non-Breeding season	Breeding season	Non-Breeding season	Breeding season	Non-Breeding season	Breeding season	Non-Breeding season
eCG + GnRH	7.33 ± 0.54^{ac}	2.43 ± 0.22^{b}	1.02 ± 0.14^{ac}	0.41 ± 0.12^{bc}	4.32 ± 0.56^{ac}	1.61 ± 0.12^{b}	0.28 ± 0.08^{ac}	1.5 ± 0.56^{bc}
eCG	4.33 ± 0.39^{bd}	2.27 ± 0.38	2.45 ± 0.25^{d}	1.16 ± 0.3^{d}	2.66 ± 0.66^{ad}	0.83 ± 0.3^{b}	2.81 ± 0.21^{d}	2.33 ± 0.46^{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.

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References

1. Al-Mola, M. K. M. A. (2008). A study of superovulation and embryo recovery in Iraqi Awassi ewes. M.Sc thesis College of Veterinary Medicine, University of Mosul, Mosul, Iraq, pp. 79-96.
2. Cognie, Y. (1999). State of art in sheep-goat embryo transfer. Theriogenology, 51: 105-116.
3. Cognie, Y.; Baril, G.; Poulin, N. & Mermillod, P. (2003). Current status of embryo technologies in sheep and goats. Theriogenology, 59: 171-188.
4. Deligiannis, C.; Valasi, I.; Rekkas, C. A. P.; Theodosiadou, E.; Lainas, T. & Amiridis, C. S. (2005). Synchronization at ovulation and fixed time intrauterine insemination in ewes. Reprod. Dom. Anim., 40: 258-272.
5. Ishwar A. K. & Memon M. A. (1996). Embryo transfer in sheep and goats: a review. Small Rum. Res., 19: 35-43.
6. Naqvi, S. M. K. & Gulyani, R. (1999). Ovarian response and embryo recovery to different superovulatory regimens in Rambouillet ewes under semi-arid conditions. Small Rum. Res., 34: 127-131.
7. Gonzalez-Bulnes, A.; Baied, D. T.; Campbell, B. K.; Cocero, M. J.; Garcia-Garcia, R. M.; Inskeep, E. K.; Lopez-Sebastian, A.; Mcneilly, A. S.; Santiago-Moreno, J.; Souza, C. J. H. & Veiga-Lopez, A. (2004). Multiple factors affecting the efficiency of multiple ovulation and embryo transfer in sheep and goats. Reprod. Fertil. Dev., 16: 421-435.
8. Jabbour, H. N.; Ryan, J. P.; Evans, G.& Maxwell, M. C. (1991). effects of season, GnRH administration and lupin supplementation on the ovarian and endocrine response of Merino ewes treated with PMSG and FSH-p to induce superovulation. Reprod. Fertil. Dev., 3: 699-707.
9. Jabbour, H. N. & Evans, G. (1991). Ovarian and endocrine responses of Merino ewes following treatment with PMSG and GnRH or PMSG antiserum. Anim. Reprod. Sci., 24: 259-270.
10. Ryan, J. P.; Hunton, J. R. & Maxwell, W. M. C. (1992). Time of ovulation in Merino ewes superovulated with PMSG and FSH-p. Reprod. Fertil. Dev., 3: 551-560.
11. Samartzi, F.; Boscos, C.; Vainas, E. & Tsakalof, P. (1995). Superovulatory response of Chios sheep to PMSG during spring and autumn. Anim. Reprod. Sci., 39: 215-222.
12. Rubianes, E; Ibara, D.; Ungerfield, R.; de Castro, T. & Carbajal, B. (1995). Superovulatory response in anestrous ewes is affected by the presence of a large follicle. Theriogenology, 43: 465-472.
13. Bettencourt, E. M.; Dettencourt, C. M.; Chagos; de Silva, J.; Ferreira, P.; Manito, C. I.; Matos, C. M.; Romao, R. J. & Rocha, A. (2008). effect of season and gonadotrophin preparation on superovulatory response and embryo quality in Portuguese Black Merinos. Small Rum. Res., 74: 134-139.
14. Walker, S. K.; Smith, D. H.; Frensham, A.; Ashman, R. J. & Seamark, R. F. (1989). The use of synthetic gonadotropin releasing hormone treatment in the collection of sheep embryos. Theriogenology, 31: 741-752.