Successful Superovulation and Non-surgical Embryo Collection in Sinai Goats

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Abstarct: Sinai goats are adapted to arid and harsh environment. There is a paucity of scientific knowledge on Sinai goats. In order to provide some information on the reproductive performance of Sinai does, twenty parous Sinai does, aged 3-4 years and weighing 20-26 kg, were synchronized for estrus using intravaginal sponges and superovulated with eCG (equine Chorionic Gonadotropin). Does were then divided to two equal parts, ten in each. The first part was treated by GnRH (Gonadotropin Releasing Hormone). By way of comparison, the other ten does were administrated with hCG (human Chorionic Gonadotropin). Animals showed estrus were mated with fertile bucks as long as females permitted the male to mount. Seven days after the last mating, embryos were collected transcervically by a non-surgical method. Does superovulated with eCG + hCG showed the onset of estrus at time later than the does superovulated with eCG + GnRH (49 vs 43 h). However, this difference was not significant. Does superovulated with eCG + hCG showed a slightly shorter interval between onset and the end of estrus (28 vs 29 h). Although all superovulated does showed estrus, only 70% of does produced embryos. The proportion was higher (80%) in does superovulated with eCG + GnRH. Ultrasonography examinations showed that although the mean number of follicles more than 5 mm in diameter was higher in does superovulated with eCG + hCG (14.7 vs 13.5), the mean number of ovulations was slightly higher in does superovulated with eCG + GnRH (8.6 vs 8.3). These differences were not significant. The mean number of embryos recovered and the mean number of transferable embryos tended to be higher in the does superovulated with eCG + GnRH compared to the does superovulated with eCG + hCG (6.5 vs 4.9 and 3.9 vs 3.4, respectively). This leads to the conclusion that Sinai goats could be superovulated with eCG and are able to provide embryos of good quality using non-surgical collection.

Keywords: Sinai goat, Superovulation, GnRH, Embryos.

INTRODUCTION

The effectiveness of superovulatory treatments is a critical factor in embryo transfer program in domestic animals. Improvement of animal populations using high genetically valuable animals may be hampered by an unsatisfactory superovulatory response and the recovery of low embryo quality. Most successful superovulation protocols in goats are based on the use of follicle stimulating hormone (FSH), which must be injected at 12-h intervals for 3 or 4 consecutive days (Armstrong *et al.*, 1983*a,b*; Pendleton *et al.*, 1992). On the other hand, because of the long half-life of eCG (Schams *et al.*, 1978), superovulation can be achieved with a single dose of eCG (Armstrong *et al.*, 1983*a,b*); this is a distinct advantage when goats are unaccustomed to handling, as is often the case with nomadic grazing in the Sinai peninsula.

Of the Sinai peninsula, in particular in its south, there are small-sized goats of the Sinai breed. The Sinai peninsula is a mountainous, arid region with a natural vegetation nutritionally poor in quantity and quality. Sinai goats are well adapted to these harsh conditions (Shkolnik *et al.*, 1972), and provide milk and meat to inhabitants. Most of the goat research conducted in Egypt to date was concerned with the common Zaraibi or Baladi goats mostly found in the valley and delta of the river Nile. There is a paucity of scientific knowledge on Sinai goats.

The present investigation, were conducted to provide some information on the possibility to superovulate Sinai goats, as a step in the way of improving their performance in their natural region.

MATERIALS AND METHODS

The study was conducted at the Experimental Farm of Faculty of Agriculture, Suez Canal University in Ismailia, Egypt (30°37′N, 32°16′E). Twenty parous Sinai does, aged 3-4 years and weighing 20-26 kg, were used in this experiment. Does were housed in semi-open pens. The same feeding conditions (N.R.C., 1981) were applied to all animals.

Estrus was synchronized with intravaginal sponges containing 60 mg of Medroxy Progesterone Acetate (MAP, Veramix®, Pharmacia & Upjohn, Orangeville, Canada) for 12 days. Does were intramuscularly injected with two luteolytic doses of 5 mg Dinoprost (Prostaglandin $F_{2\alpha}$, Lutalyse™, Pharmacia, Puurs, Belgium) at 12 h interval at the day of sponge removal. In order to obtain multiple ovulations (Tsunoda and Sugie, 1989; Yuswiati and Holtz, 1996), all does were intramuscularly injected at sponge removal with 750 IU eCG (equine Chorionic Gonadotropin, Folligon®, Intervet, Boxmeer, Holland). After 45 to 48 h, does were divided to two equal parts. The first ten does were treated by 0.004 mg Buserlin (Gonadotropin Releasing Hormone - GnRH, Receptal®, Intervet, Boxmeer, Holland). By way of comparison, the other ten does were administrated with 500 IU hCG (human Chorionic Gonadotropin, Ovogest®, Intervet, Unterschleissheim, Germany). Animals showed estrus were mated with fertile bucks at 12 h interval as long as females permitted the male to mount. The time of onset of estrus and its duration were recorded to determine the number of ovulations in superovulated does, ultrasonography examinations were performed twice daily (at 12 h interval) from the day of sponge removal until 48 h after the end of estrus. Ultrasonographic observation was conducted using a real-time B-mode scanner (Honda

HS-1500V, Honda Electronics, Japan) equipped with a 7.5 MHz linear-array transducer (Honda HLV-375M) for transrectal ultrasonography. The rectal transducer was modified by taping plastic rod along its dorsal border to elongate and improve its intra-rectal use in small ruminants. The transducer with carboxymethylcellulose gel (Elk Corporation, New Jersey, U.S.A.) was introduced into the rectum until the bladder appeared on the screen. Then, the probe was pushed and rotated towards the right and the left side to find both ovaries. Ovulation was defined by disappearance of follicles with 5 mm or more in diameter (Fig. 1) that had been found on the observation previous (De Castro et al., 1998; Tom et al., 1998).

Seven days after the last mating, embryos were collected transcervically by a non-surgical method as described by Suyadi et al. (2000). Briefly, superovulated does were intramuscularly injected with a luteolytic dose of 5 mg $PGF_{2\alpha}$ at 24 h before embryo collection. Embryo collection was performed in unanesthetized does in a standing position. One person restrained the doe in standing position by its neck. For viewing and grasping of the lip of the os cervix, the vagina must be dilated by insertion of a duck-billed speculum into the vagina and grasping of the lip of the external os of the cervix with the Allis forceps (Surgicon, J-20-290, 25 cm, Pakistan). The flushing catheter (Rüsch® No. 12, 40 cm long, Kernen, Germany) was then inserted into the os cervix and passed through the cervical canal deep into one uterine horn by exertion of gentle pressure. The tip of the catheter may be directed either into the left or right uterine horn with a finger in the vaginal fornix. Once the catheter was introduced into the desired uterine horn, 20 ml PBS flushing medium (Dulbecco's Phosphate Buffered Saline) at 39°C was infused into the free end with a 20-ml syringe. After infusion of 20 ml of medium, the syringe was disconnected from the catheter to permit spontaneous back-flow of the medium. The medium was collected in a 50 ml test tube. This process was repeated 10 times. The catheter was then introduced into the other horn and another 10 flushings were conducted. Thereafter, the catheter was positioned back into the right uterine horn for 5 more flushings and again into the left horn for another 5 flushings. The collected medium was poured into a petri dish (94 x 16 mm) to be examined under a stereo microscope at 20 to 40x. With the aid of a unopette (20 µl capacity, Becton-Dickinson, Rutherford, New Jersey, USA), embryos were picked out and transferred to a small petri dish (35 x 10 mm) containing 2 ml of M2 medium (Hogan et al., 1986), then examined under a stereo microscope at 40x for stage of development and morphological integrity. The transferable embryos rate was estimated as the percentage of transferable embryos recovered relative to the number of total embryos or ova recovered.

Statistical analysis was performed using the general linear models (GLM) procedure of the SAS statistical package 6.12 (SAS Institute, 1998). Duncan's test (1955) was used to compare the differences between groups.

RESULTS AND DISCUSSION

Generally, the problem with superovulatory treatment is the large degree of variation in the number of transferable embryos among individuals in the same species (Herrler et al., 1988; Kim et al., 1988; Plus-Kleingeld et al., 1991). Also, goats superovlated with eCG exhibited high variation in ovarian response and large number of unovulated follicles compared to FSH-treated goats (Armstrong et al., 1983b; Nowshari et al., 1992). Therefore, in the present study, effect of hCG (control) and GnRH administrations in superovulatory regimen with eCG on ovulatory response and embryo yield was investigated.
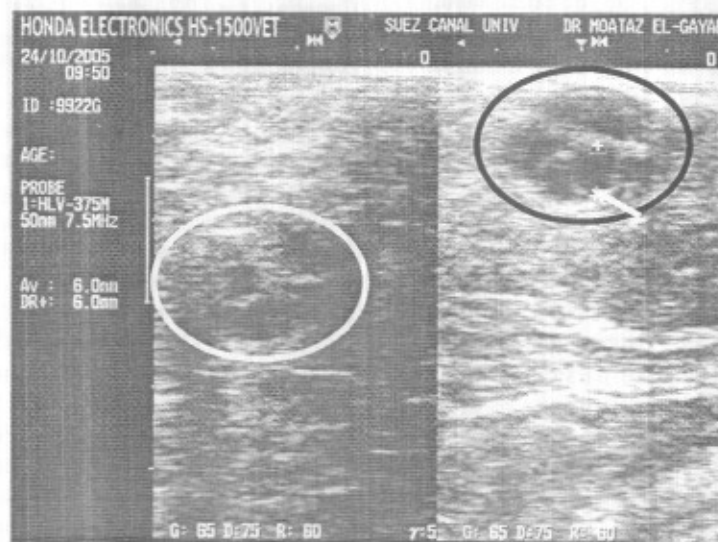

Figure (1): An ultrasonograph of ovaries in a doe superovulated with eCG observed by trans-rectal transducer. Left is an image of the left ovary (white ring) and right is the right one (black ring) with follicles. Note a follicle with 6 mm in diameter (white arrow).

As indicated in Table 1, all superovulated does showed estrus. The onset of estrus is expressed as time interval (h) from implant removal to the appearance of the first signs of estrus. Does superovulated with eCG + hCG showed the onset of estrus later than the does superovulated with eCG + GnRH (49 vs 43 h). However, this difference was not significant (P < 0.05). Does superovulated with eCG + hCG showed a slightly shorter interval between onset and the end of estrus (28 vs 29 h). Onset and duration of estrus in synchronized goats depend on many factors i.e. dose and type of drug, form and route of administration, duration of application, phase of estrus cycle, breeding season and nutrition (Umberger et al., 1994; Kafi and Mcgowan, 1997). Although all superovulated does showed estrus, only 70% of does yielded embryos. The proportion was higher (80%) in does superovulated with eCG + GnRH. Flores-Foxworth et al. (1992) reported that of the seventy one does that were superovulated with eCG, 18 (25%) did not show estrus, 6 (9%) showed estrus but did not ovulate and 9 (13%) showed early regressing corpora lutea. Superovulated does with eCG frequently suffer from premature luteal regression (Armstrong et al., 1982a, 1982b, 1983a; Battye et al., 1988), which results in the loss of most embryos before collection is attempted on Day 6 or 7 after the onset of estrus (Armstrong et al., 1982a, 1982b, 1983b, 1987). Different treatments have been developed to prevent early luteal regression in goats superovulated with eCG, including the use of prostaglandin F2α inhibitors (Battye et al., 1988) and the administration of to luteinize estradiol-producing follicles (Smith and Murphy, 1984; Battye et al., 1988).

As shown in Table 2, although the mean number of follicles more than 5 mm in diameter was higher in does superovulated with eCG + hCG (14.7 vs 13.5), the mean number of ovulations was slightly higher in does superovulated with eCG + GnRH (8.6 vs 8.3). These differences were not significant (P < 0.05). The yield of transferable embryos after superovulation is more important for a successful embryo transfer than the number of ovulations induced in each individual animal. The mean number of embryos recovered and the mean number of transferable embryos tended to be higher in the does superovulated with eCG + GnRH compared to the does superovulated with eCG + hCG (6.5 vs 4.9 and 3.9 vs 3.4, respectively). These means achieved were as high or even higher than previously reported in other studies (Goel and Agrawal, 1990; Yuswiati and Holtz, 1996). The response of does superovulated with eCG, in the present investigation, were not strongly influenced by the presence of GnRH in superovulation regimen. However, the administration of GnRH around the time of estrus onset has been shown to reduce the presence of large unovulated follicles at the time of embryo collection in goats that were superovulated with FSH (Akinlosotu and Wilder, 1993; Krisher et al., 1994). The ovulatory failure may be due to asynchronous follicular growth (Pendleton et al., 1992), which could result in lack of LH receptors in those follicles that are in earlier stages of development at the time of the endogenous preovulatory LH peak (Armstrong et al., 1982b; Pendleton et al., 1992). Furthermore, the continued presence of eCG in the circulation, due to its long half-life, could induce the growth of new estradiol-producing follicles after the first wave of superovulation has taken place (Pendleton et al., 1992).

This leads to the conclusion that since Sinai goats could be superovulated with eCG and are able to provide embryos of good quality using non-surgical collection, we consider it is important to make the best of this breed attempting to improve their productive performance, by selective breeding, rather than venturing too hastily into crossbreeding.

Table (1): Response of Sinai goat does to different superovulation treatment regimes.

Treatment	Does treated	Does in estrus	Onset of estrus* (Mean ± SEM)	Duration of estrus (Mean ± SEM)	Does yielding embryos	
					n	%
eCG + hCG	10	10	49 h ± 5.1	28 h ± 2.2	6	60[a]
eCG + GnRH	10	10	43 h ± 4.6	29 h ± 1.8	8	80[b]

[a,b] Within columns values with different superscripts differ (P < 0.05).
*The onset of estrus is expressed as time interval (h) from implant removal to the appearance of the first signs of estrus.

Table (2): Ovulatory response to different superovulation treatment regimes in Sinai goat does.

Treatment	Follicles > 5 mm per doe		Ovulations per doe			Embryos per doe flushed		Transferable embryos per doe flushed		
	Mean	SEM	Mean	SEM	%	Mean	SEM	Mean	SEM	%
eCG + hCG	14.7	1.8	8.3	0.9	56.5	4.9[a]	1.9	3.4	1.6	69.4
eCG + GnRH	13.5	2.2	8.6	1.3	63.7	6.5[b]	2.1	3.9	1.6	60.0

[a,b] Within columns values with different superscripts differ (P < 0.05).

REFERENCES

Akinlosotu B. A. and C. D. Wilder (1993). Fertility and blood progesterone levels following LHRH induced superovulation in FSH-treated anestrons goats. Theriogenology 40: 895-904.

Armstrong D. T., A. P. Pfitzner and R. F. Seamark (1982a). Ovarian responses and embryo survival in goats following superovulation and embryo transfer. Theriogenology 17: 76 (Abstr.).

Armstrong D. T., A. P. Pfitzner, K. J. Porter, G. M. Warnes, P. O. Janson and R. F. Seamark (1982b). Ovarian response of anoestrous goats to stimulation with pregnant mare serum gonadotrophin. Anim. Reprod. Sci. 5: 15-23.

Armstrong D. T., A. P. Pfitzner, G. M. Warnes, M. M. Ralph and R. F. Seamark (1983a). Endocrine responses of goats after induction of superovulation with PMSG and FSH. J. Reprod. Fertil. 67: 395- 401.

Armstrong D. T., A. P. Pfitzner, G. M. Warnes and R. F. Seamark (1983b). Superovulation treatments and embryo transfer in Angora goats. J. Reprod. Fertil. 67: 403-10.

Armstrong D. T., D. J. Kiehm, G. M. Warnes and R. F. Seamark (1987). Corpus luteum (CL) failure and embryonic loss in superovulated goats. Theriogenology 27: 207 (Abstr.).

Battye K. M., R. J. Fairclough, A. W. N. Cameron and A. O. Trounson (1988). Evidence for prostaglandin involvement in early luteal regression of the superovulated nanny goats (Capra hircus). J. Reprod. Fertil. 84: 425-30.

De Castro T., E. Rubianes, A. Menchaca and A. Rivero (1998). Ultrasonic study of follicular dynamics during the estrous cycle in goats. Theriogenology 49: 399 (Abstr.).

Duncan D. B. (1955). Multiple range and multiple F test. Biometrics 11: 1-42.

Flores-Foxworth G., B. M. BcBride, D. C. Kraemer and L. C. Nuti (1992). A comparison between laparoscopic and transcervical embryo collection and transfer in goats. Theriogenology 37: 213 (Abstr.).

Goel A. K. and K. P. Agrawal (1990). Superovulation and embryo collection in Jumnapari goats. Theriogenology 33: 232 (Abstr.).

Herrler A., J. F. Beckers, J. Viviers-Donnay and H. Niemann (1988). Purified FSH supplemented with defined amounts of LH for superovlation in dairy cattle. Theriogenology 29: 260 (Abstr.).

Hogan B., F. Costantine and E. Lacy (1986). Manipulating the mouse embryo: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Kafi M. and M. R. Mcgowan (1997). Factors associated with variation in the superovulatory response of cattle. Anim. Reprod. Sci. 48: 137-157.

Kim H. N., J. D. Roussel, S. H. Pool and R. A. Godke (1988). The effect of a commercially-available purified FSH and bovine anti-PMSG serum on the superovulation of dairy heifer. Theriogenology 28: 267 (Abstr.).

Krisher R. L., F. C. Gwazdauskas, R. L. Page, C. G. Russell, R. S. Canseco, Sparks AET., W. H. Velander, J. L. Johnson and R. E. Person (1994). Ovulation rate, zygote recovery and follicular populations in FSH-superovulated goats treated with PGF2 and/or GnRH. Theriogenology 41: 491-498.

Nowshari A. M., E. Yuswiati, M. Puls-Kleingeld and W. Holtz (1992). Superovulation in peripubertal and adult goats treated with PMSG and FSH. In: Lokeshwar RR (ed.), Recent Advances in Goat Production. Nutan Printed, New Delhi, India: 1235-1243.

NRC (1981). National academy of sciences, national research council. Nutrient requirements of goats, Washington, D.C.

Pendleton R. J., C. R. Youngs, R. W. Rorie, S. H. Pool, M. A. Memon and R. A. Godke (1992). Follicle stimulating hormone versus pregnant mare serum gonadotropin for superovulation of dairy goats. Small Rumin. Res. 8: 217-224.

Puls-Kleingeld M., E. Yuswiati, A. M. Nowshari, J. F. Beckers and W. Holtz (1991). Effect of FSH/LH ratio and treatment schedule on superovulatory response in Boer goats. J. Reprod. Fert. Suppl. 34: 308 (Abstr.).

SAS (1998). Statistical Analysis System, Version 6.12. Cary, NC, SAS Institute Inc.

Schams D., C. H. Menzer, E. Schallenberger, B. Hofmann, J. Hahn and R. Hahn (1978). Some studies on PMSG and on endocrine response after application for superovulation in cattle. In: control of reproduction in the cow, Sreenan JM. (Ed.). Verlag Martinus Nijhoff, the Hague, 122-143.

Shkolnik A., A. Borut and J. Choshniak (1972). Water economy of the Bedouin goat. Symp. Zool. Soc. Lond. No. 31, 229-242.

Smith C. L. And C. N. Murphy (1984). A modified antegrade uterine flush for recovery of ova in the ewe. In: Proceedings of the 10th International Congress on Animal Reproduction and Artificial Insemination, June 10–11. Urbana Champaign, University of Illinois: 242–5.

Suyadi, Sohnrey B. And W. Holtz (2000). Transcervical embryo collection in Boar goats. Small Rumin. Res. 36: 195-200.

Tom J. W., R. A. Pierson and O. J. Ginther (1998). Quantitative echostructure analysis of bovine ovarian follicles. Theriogenology 50: 339-346.

Tsunoda Y. and T. Sugie (1989). Superovulation in nonseasonal japanese native goats: with special reference to the developmental progression of embryos. Theriogenology 31: 991-996.

Umberger S. H., G. Jabbar and G. S. Lewis (1994). Seasonally anovulatory ewes fail to respond to progestogen treatment in the absence of gonadotrophin stimulation. Theriogenology 42: 1329-1336.

Yuswiati E. and W. Holtz (1996). Superovulation with different exogenous gonadotropins in peripubertal goats. Indian J. Anim. Sci. 66: 131-133.

نجاح التبويض الفائق والجمع الغير جراحي للأجنة في الماعز السيناوي

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تتميز الماعز السيناوي بتأقلمها مع الظروف البيئية القاسية. المعلومات العلمية عن هذه الماعز قليلة وقد تكون نادرة. لذلك كان الغرض من هذا البحث هو معرفة بعض المعلومات عن الأداء التناسلي لإناث الماعز السيناوي في حالة دفعها للتبويض الفائق باستخدام هرمون جونادوتروبين سيرم الأفراس الحوامل (eCG).

استخدم في هذه التجربة عشرين انثي ماعز سيناوي سبق لها الولادة ويتراوح عمرها ما بين ٣ - ٤ سنوات وبمتوسط وزن ما بين ٢٠- ٢٦ كجم، وقسمت إلي مجموعتين متساويتين تم تزامن شياعها ودفعت للتبويض الفائق باستخدام ال eCG. في المجموعة الأولي تم دراسة تأثير استخدام الهرمون المحرر للجونادوتروبينات (GnRH) مع هرمون ال eCG علي الاستجابة للتبويض ومحصول الأجنة. وعلي سبيل المقارنة استخدم الهرمون الكريوني الآدمي المنشط للغدد الجنسية hCG مع ال eCG في المجموعة الثانية. وقد تم جمع الأجنة من إناث الماعز المعاملة بالطريقة الغير جراحية في اليوم السابع بعد التلقيح.

أظهرت النتائج أن إناث الماعز في المجموعة الثانية بدأت الدخول في الشياع متأخرة عن المجموعة الأولي (٤٩ مقابل ٤٣ ساعة). بينما كان طول فترة الشياع في المجموعة الثانية أقصر قليلا عن الأولي (٢٨ مقابل ٢٩ ساعة). وعلي الرغم من أن جميع الإناث ظهر عليها علامات الشياع إلا أن ٧٠% فقط من الإناث أنتجت أجنة. وقد تفوقت المجموعة الأولي في نسبة عدد الإناث المنتجة للأجنة (٨٠%). كذلك فقد أظهر الفحص باستخدام جهاز الموجات فوق الصوتية المصورة أن متوسط عدد الحويصلات المبيضية التي قطرها اكبر من ٥ مم كان أعلي في إناث المجموعة الثانية (١٤,٧ مقابل ١٣,٥)، علي الرغم من أن متوسط عدد التبويضات كان أعلي بشكل طفيف في إناث المجموعة الأولي (٨,٦ مقابل ٨,٣). أما فيما يتعلق بالأجنة فإن متوسط عددها سواءاً الإجمالي أو القابلة للنقل (عالي الجودة) كان أعلي في المجموعة الأولي بالمقارنة بالمجموعة الثانية (٦,٥ مقابل ٤,٥ و ٣,٩ مقابل ٣,٤، علي الترتيب).

ويستنتج من هذه الدراسة أمكانية نجاح دفع إناث الماعز السيناوي للتبويض الفائق باستخدام هرمون جونادوتروبين سيرم الأفراس الحوامل (eCG) بشكل نتج عنه انتاج اجنة عالية الجودة باستخدام طريقة الجمع الغير جراحي.