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Methodology of Using eCG or FSH Regimens for Multiple Ovulations and Embryo Recovery by Assisted Reproductive Technologies in Sheep

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Abstract

This study aimed to profit the assisted reproductive technologies in sheep presented two techniques for multiple ovulations and embryo recovery. Multiparous ewes of Egyptian Barki (N=15) and Australian Dorper (N=20) were used in the first and second techniques, respectively. Technique I was performed in Faculty of Veterinary Medicine, Sadat city, Menufia province, Egypt during the period from August to February, while technique II in Werribee, Victoria, Melbourne City Australia during the period from March to April. The technique I (20-21 days) based on using intravaginal progesterone (P_4)-sponges for fourteen days, equine chorionic gonadotropin and flushing of embryos under local anesthesia 5 days after mating. However, the technique II (18 days) based on using intravaginal P_4-controlled internal drug release silicon devices for twelve days with six injections of the follicle-stimulating hormone (Folltropin; FSH), eCG and surgical flushing of embryos under general anesthesia four days after laparoscopic intrauterine insemination. The recovery rate of the harvested morulae and blastocysts in relation to the developed corpora lutea were 38.2 and 24.4 % in the technique I, respectively, while they were 40.8 and 25.8 % in the technique II, respectively. Moreover, the percent of unfertilized oocytes and lost recovered structures, those of early embryonic death absorbed or lost during flushing in the technique I were 18.2 and 31.6 %, respectively, while they were 7.3 and 13.7 % in the technique II, respectively. In conclusion, the two presented techniques showed variable results of recovered embryos, but the harvested embryos produced by ewes younger than 6 years old were higher than 38 %.

Keywords: Barki, Embryo, MOET, Dorper, Sheep, Superovulation.

Introduction

The Barki and Dorper sheep are the highest sheep breeds for meat production in their habitats, Egypt [1] and Australia [2], respectively. Superovulation or the multiple ovulations for embryo transfer (MOET) are effective means of increasing the contribution of superior females to breeding programs for embryo biotechnology. Although such biotechnology is applied in many domestic species, it is still facing many problems such as the unpredictable rates of ovulation and transferable embryos. It is known that factors such as breeds [3], nutrition [4], season [5], photoperiod [6], oocyterian status [7], gonadotrophin preparation and repeated superovulation [8] affect superovulation as well as the quality of produced embryos. Few studies were performed in Egypt with an easily applied protocol for MOET such as equine chorionic gonadotropin (eCG) for superovulation. However, the problem with repeated use of eCG is the humoral response to this gonadotropin and production of anti-eCG antibodies [9], that possibly reflects a negative impact on fertility and reproductive performance. Our study aimed to present two different easily applied protocols for multiple ovulations and embryo recovery in two different sheep breeds.

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Material and methods

Animals

A total of fifteen Barki female sheep were used for the technique I in the farm of the Faculty of Veterinary Medicine, Sadat City, Menufia, Egypt, during the period from August to February (Egyptian breeding season), while twenty Dorper sheep were used for the technique II in the farm of Animal Production, Werribee, Melbourne, Australia during the period from March to April (Australian breeding season). Animals of both techniques were clinically healthy and non-lactating multiparous aged between 2-5 years with average body weight of 40 to 50 kg. Sheep were continuously provided with water, allowed for grazing ad libitum and provided with concentrates 1.25 kg/ animal/ daily. They were repeatedly used for superovulation as donors.

Hormonal treatment, anesthetic drugs and cryoprotectant media

Hormonal drugs including intravaginal sponges saturated with fluorogestone acetate (FGA) with a dose of 40 mg (Chronogest, Intervet, Egypt) were placed in the vagina of ewes for 14 days and ewes were superovulated with eCG (Folligon, Intervet Co., Egypt) at a dose rate of 1200 IU. The GnRH injectable solution (0.0042 mg Buserelin acetate per 1 mL solution equivalent to 0.004 mg Buserelin, Receptal, Intervet Co., Egypt) was given intramuscularly at the day of mating. The controlled internal drug-release (CIDR) (Pfizer Animal Health) silicon-coated devices containing 0.3 g of progesterone/device were inserted intravaginally. The flushing solution was Dulbecco's phosphate buffered saline (PBS) supplemented with 1.0 % sheep serum in addition to antibiotics (penicillin and streptomycin). The pH adjusted to 7.2-7.6 with osmotic pressure 270 - 310 mOs. Anesthetic drugs such as xylazine (Xyla-ject, Adwia Pharmaceuticals Co. Egypt) was used as a systemic tranquilizer, 0.5 % lidocaine hydrochloride (Sigma Co., Egypt) was applied for local infiltrative anesthesia and thiopentone sodium for general anesthesia (Abbott Australasia, Kurnell, N.S.W., Australia). The cryoprotectant ethylene glycol (EG) was used for Dorper sheep embryos freezing (EMCARETM, 1.5 M 20/Box).

Technical design

Technique I (Sponge-Based Superovulation protocol)

A total of fifteen Barki multiparous ewes were used. Hormonal treatments were including intravaginal sponges impregnated with FGA 40 mg were placed in the vagina of ewes for 14 days and ewes were superovulated with eCG at a dose of 1200 I.U. After withdrawal of sponge, the animals were intramuscularly injected with GnRH (1 mL/ewe) and the estrus was checked using a fertile ram twice daily at 06:00 and 18:00 h for mating. The ewe was considered in estrus if they standing to be mounted by the ram (Technique I, Figure 1).

Technique II (CIDR-Based Superovulation protocol)

A total of twenty Dorper multiparous ewes were used. Hormonal treatments were including intravaginal insertion of CIDR devices on day zero at the morning and, FSH (containing folltropin, Bioniche Animal Health USA, Inc. 20 mL vial contains FSH equaling 400 mg NIH-FSH-P1) was injected once at the day 10^{th} (4 mL at 04:00 P.M.), twice at the day 11^{th} (3 mL at 08:00 A.M. and 2 mL at 04:00 P.M.) and twice again at the day 12^{th} (2 mL at 08:00 A.M. and 1.5 mL at 04:00 P.M.). Also, at the day 12^{th}, CIDR devices were withdrawn and 400 I.U. of eCG was administered. At the day 13^{th}, FSH was injected again in a dose of 1.5 mL at 08:00 A.M. All injections of FSH and eCG were given by I.M. injection (Technique II, Figure 2).

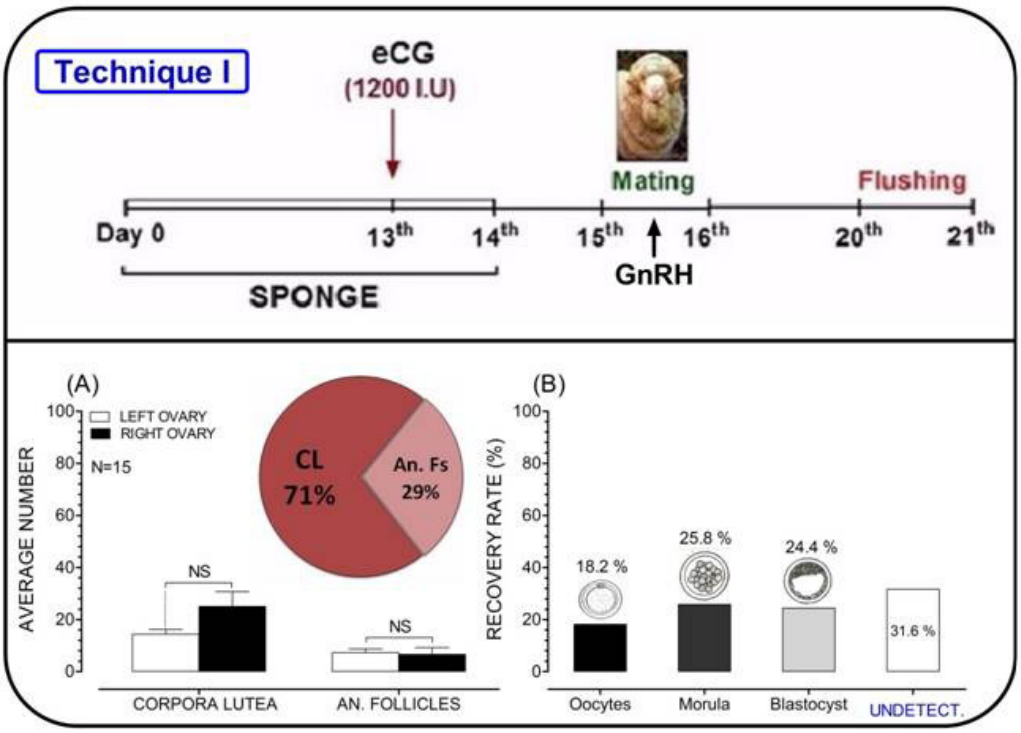

Figure 1: Technique I; an estrus synchronization and superovulation regimen in Egyptian Barki ewes (N=15). Intravaginal progesterone-saturated sponge was inserted for 14 days. Equine chorionic gonadotropin (eCG; 1200 I.U) was intramuscularly (I.M) injected at Day 13th. Estrus and mating were observed and applied during the period from 15th to 16th days. Surgical flushing of embryos under local anesthesia was applied during the period from 20th to 21th, 5 days after mating. (A) The average number of corpora lutea and the anovulatory follicles that counted after surgical laparotomy in response to the superovulation regimens. (B) The recovery rate of unfertilized oocytes and those divided embryos; morulae and blastocysts in relation to the number of corpora lutea. The undetected structures include those oocytes or embryos that had been lost or not flushed.

The procedures of semen collection for the laparoscopic insemination are shown in Figure 3. At the day 14th, semen was collected from fertile Dorper rams at 06:00 A.M using electroejaculator with maximum collection of twice weekly per ram. The obtained semen ejaculate (1-2 mL/ram) was examined macroscopically and microscopically for color, quality and motility. The ejaculate was diluted by triladyl diluent (1:6) (Minitub, Tiefenbach, Germany). The extender, triladyl was added carefully by Pasteur pipette to the tube wall that containing the semen. The extended semen was examined again by the low power microscope (x40) for gross motility. The straws were filled by the extended semen as 0.25 mL each ready for direct use. The superovulated ewes were intrauterine inseminated using laparoscope at 08:00 A.M after disinfecting the area in front the udder by iodine. Each animal was intramuscularly injected with Oxytetracyclin (5 mL) as a prophylactic antibiotic.

Flushing of embryos

The procedure was similar in both techniques but differed in the anesthesia as the technique I was performed under local anesthesia but the technique II was performed under general anesthesia. The treated ewes designated for flushing of embryos were fasted over the night of the day 17th. On the morning of the day 18th, the animals were prepared for surgical flushing as the following: a) shaved the area in front of the udder and thoroughly disinfected by spraying iodine followed by alcohol, b) anesthesia; for technique I, the animals were anesthetized by intravenous injection of xylazine 0.22 mg/kg BW (Xylagect 0.05- 0.15 mL) as a tranquilizer and the mid-lateral site for laparotomy was locally anesthetized by infiltration with 4-6 mL of 0.5 % lidocaine hydrochloride in the incision area, while for technique II, the animals were anesthetized by I.V. injection of thiopentone sodium 20 mg/kg BW, c) 1-2 cm incision was

performed on the para-midline space in front of the udder to allow the exteriorization of the uterine horns out of the animal, d) the oocyterian response was qualified by counting the functional corpora lutea (CL) and the anovulatory follicles according to the characteristic morphology of each structure, e) uterine flushing; embryos were recovered as described by Smith and Murphy [10], near the base of the uterine horn a two-way pediatric Foley catheter was inserted through an stabbing opening made by forceps and the cuff was inflated with air by the aid of 5-mL syringe. An I.V. human cannula was being inserted in the uterine horn just near to the utero-tubal junction and being attached to a flushing solution containing syringe which pushed carefully (20 - 40 mL for each horn) for flushing of the recovered embryos in a Petri dish. Finally, to avoid the post-operative adhesions inside the abdomen, flushing about 500 mL of saline into the abdomen was done before closure (Figure 4).

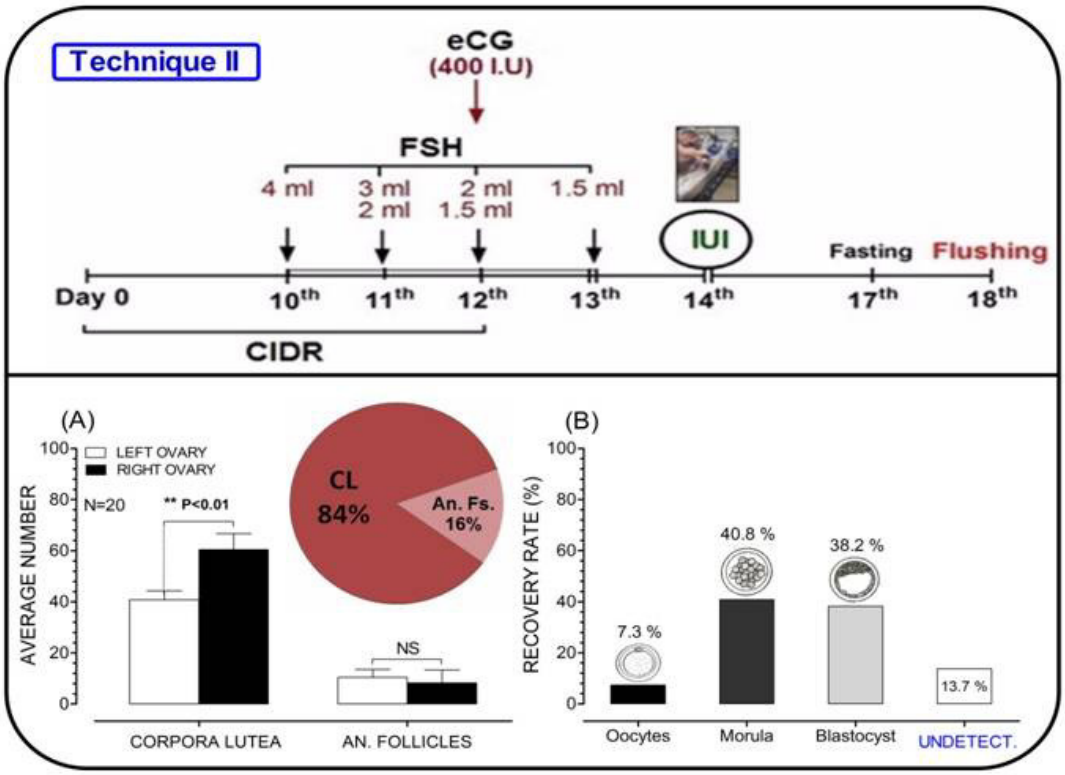

Figure 2: Technique II; (A) Ovsynch and superovulation regimen in Australian Dorper ewes (N=20). Intravaginal controlled-internal drug release (CIDR) was inserted for 12 days with I.M injection of eCG (400 I.U) at the Day 12th after removal of CIDR. Folltropin (FSH) was injected I.M 6 times in 4 subsequent days as shown from Day 10th to 13th. Intrauterine insemination (IUI) was applied by the Day 14th using the laparoscope and surgical flushing of embryos by the Day 18th. Both IUI and embryo flushing were performed under general anesthesia. Other explanations are given in Figure 1.

Figure 3: The procedures for semen collection from Dorper rams using electroejaculator, extention of semen and microscopic examination and intrauterine insemination using the laparoscope were shown respectively in images A-C, C-E and F.

Evaluation of embryos

The recovered structures, unfertilized oocytes and embryos, were evaluated microscopically for the developmental stage and quality considered the morphological criteria under a stereo microscope according to Lindner and Wright [11]. The recovered structures were classified into a) unfertilized oocytes (not cleaved), b) degenerated embryos and c) transferable good embryos, as intact compact morula, early blastocyst and expanded blastocyst (Figure 5).

Cryopreservation of the transferable embryos

Based on Leibo [12] and Youngs [13], the transferable embryos including the morulae and blastocysts were prepared for slow freezing in liquid nitrogen after cryoprotection using ethylene glycol (EG) as a hypertonic cryoprotective agent (CPA) (1.4 – 1.5 M). The embryos were subjected to EG and holding solution (HS) in different concentrations as isotonic medium; 5-6 min in a drop of 1 third EG + 2 thirds HS, 5-6 min in a drop of 2 thirds EG + 1 third HS and finally 10 min in a drop of EG only. Using the two-air columns method, the 0.25 mL straws were filled by the embryos and transferred to the cryobath freeze control machine for gradual freezing up to - 35 ºC with regard to seeding after 5 min of exposure to liquid nitrogen vapor. After reaching the desired degree, the straws were transferred to goblets and canisters immersed in liquid nitrogen at -196 ºC (Figure 5).

Statistical analysis

The oocyterian activity including production of the corpora lutea and anovulatory follicles produced by the right and left oocytes in response to their specific technical protocol of superovulation were statistically analysed by two-way ANOVA and confirmed by Bonferroni as a post-hoc test. All data were represented as the mean ± S.E.M. The differences were considered significant at $P<0.05$ by using Graph Pad Prism software program (V.5.01, SAN DIEGO USA, 2007).

Results

The technique I procedures that depended on P_4-sponge/eCG-based regimen for superovulation are shown in Figure 1. The average numbers of corpora lutea and the anovulatory follicles that were counted after surgical laparotomy in response to that regimen are shown in Figure 1A. The recovered oocytes and embryos; morulae and blastocysts are shown in Figure 1B. The right

oocyterian response to the superovulation regimen was highly active compared to the left one. The total number of corpora lutea formed in response to eCG-injections was higher than those of anovulatory follicles (39.4 ± 7.38 vs. 13.8 ± 4.11, respectively) that constituted 71 vs. 29 %, respectively. The average number of corpora lutea produced by the right oocyterian response tended to be higher than those produced by the left one in response to the eCG-based protocol (25.0 ± 5.6 vs. 14.4 ± 1.74, respectively), and that the number of anovulatory follicles recorded between both oocyterian activities showed non-significant differences (Figure 1A). The recovery rate of unfertilized oocytes was 18.2 %, and those divided embryos; morulae and blastocysts were 25.8 and 24.4 %, respectively. However, the percent of undetected structures in relation to the number of corpora lutea was 31.6 % (Figure 1B).

Figure 4: The procedures of the surgical flushing of embryos 5 days after IUI of Dorper ewes were shown in respective images from A-P. Images A-C, the preparation of surgical area; Image D, intravenous injection of thiopentone (5 mL for anesthesia);, Images E-G, the outside exploration of uterus, oocytery and oviducts; Images H-J, the insertion of Foley catheter at the base of uterine horns; Images J-K, the insertion of human canula at the tip of the uterotubal junction; Images L and M, the process of embryo flushing was performed by inflation of air in the catheter cuff and infusion of the solution media in the canula to be received in the Petri dish; Images N-P, the uterus and surgical opening were washed by saline to prevent adhesions before closure by single stitshes using silk.

The technique II procedures that depended on P_4-CIDR/FSH/eCG-based superovulation regimen were shown in Figure 2. The average numbers of corpora lutea and the anovulatory follicles that were counted in response to that regimen were shown in Figure 2A. The recovered oocytes and embryos including morulae and blastocysts were shown in Figure 2B. The right oocyterian response to the superovulation regimen was highly active compared to the left one. The total number of corpora lutea formed in response to

FSH/eCG-injections was higher than those of anovulatory follicles (101.2 ± 9.76 vs. 18.6 ± 8.22, respectively) that constituted 84 vs. 16 %, respectively. The average number of corpora lutea produced by the right oocyterian activity was significantly higher than those produced by the left one in response to the FSH/eCG-based protocol (60.4 ± 6.3 vs. 40.8 ± 3.5) ($P<0.01$) and that the number of anovulatory follicles recorded between both oocyterian activities showed non-significant differences (Figure 2A). The recovery rate of unfertilized oocytes was 7.3%, and those divided embryos; morulae and blastocysts were 40.8 and 38.2 %, respectively. However, the percent of undetected structures in relation to the number of corpora lutea was 13.7 % (Figure 2B).

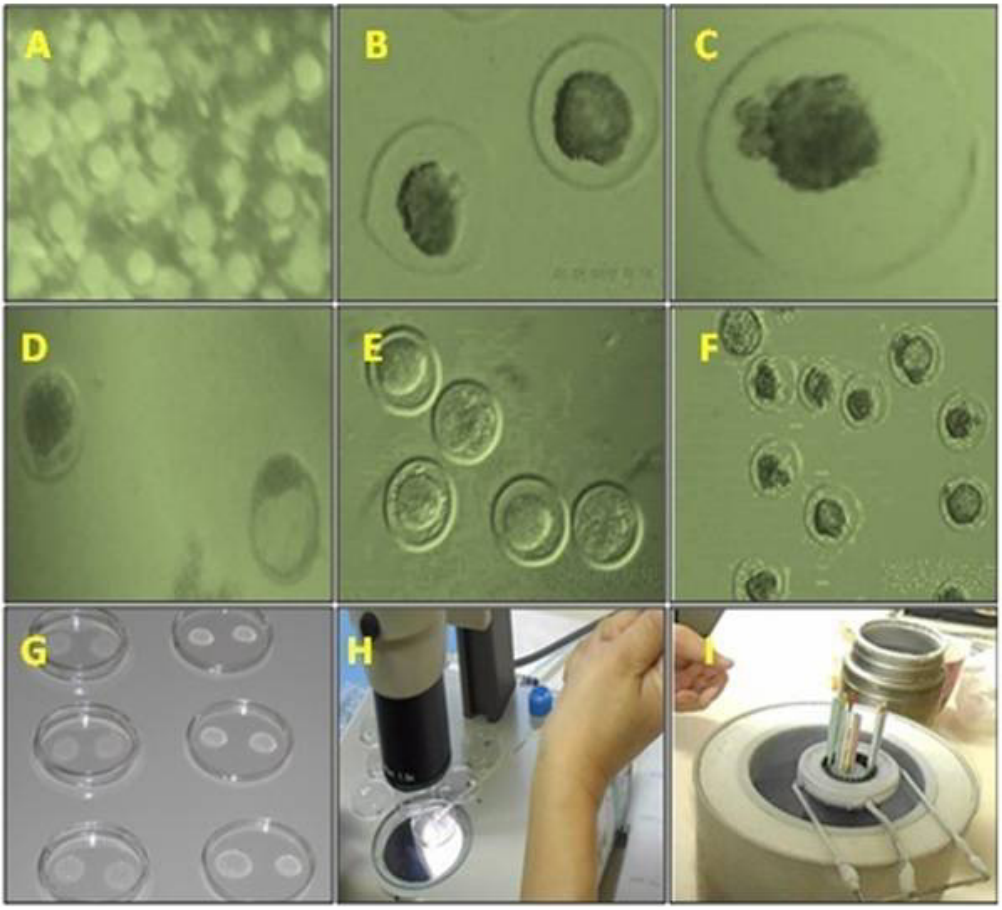

Figure 5: Images A-I, microscopic examination of recovered embryos, selection, cryopreservation and gradual freezing of embryos-containing straws in liquid nitrogen; Images B and C, undivided oocytes; Image D, transferable morula to right, and blastocyst to left; Image E, untransferable degenerated embryos; Image F, transferable blastocysts; Images G and H, cryopreservation of the selected embryos in the cryoprotectant ethylene glycol and holding solution; Image I, gradual freezing of the embryos-containing straws using cryobath lquid nitrogen freeze control.

Discussion

Although the oocyterian activities of young females can respond to the given external gonadotropins, they may respond slower to superovulation and known to be highly sensitive to the negative effects of steroids [14]. Although, the best embryo production was found around six years of age [14, 15], our results of the embryo production was evaluated in ewes of variable ages with maximum five years old giving a probability of improving embryos production by age. For those reasons The eCG and FSH are generally included in the MOET in higher doses. The eCG is preferred than FSH due to its lower cost, easy availability and.easily administered, usually with a single injection of 1500-2000 I.U, but its

superovulatory response is variable and usually lower than that of FSH [16]. There were several problems associated with PMSG-induced superovulation such as high number of anovulatory follicles, early regression of corpus luteum and potential risk of embryo expulsion.

The long half-life of eCG caused over-stimulation of follicular growth, even after ovulation, which induced early luteal regression. The end result is a decline of circulating progesterone concentration before embryo collection and hence a low embryo quality and embryo survival rate being obtained [17]. The administration of progesterone (P_4) alone or P_4 combined with eCG or FSH affects the endocrine activity and showed a great variety in the ovsynch, onset time of estrus and ovulations [18] in addition to the premature luteal regression [19]. In the present study, the different treatment forms of P_4-eCG or P_4-FSH-eCG affected the oocyterian response of the superovulated ewes and therefore, the recovered oocytes and embryos.

The recovery rate of the harvested morulae and blastocysts in relation to the developed corpora lutea and ovulatory follicles in response to P_4-FSH-eCG of the technique II were high that was in agreement to several studies [20]. Furthermore, the percent of unfertilized oocytes and lost recovery structures of early embryonic death absorbed or lost during flushing and the total viable transferable embryos were variable. The eCG in a dose of 1200 I.U showed satisfactory results, but higher doses caused side effects as higher anovulatory follicles and lower recovery rate, which were in agreement with Holtz *et al*. [21]. The current results dealing with those in response to using eCG/FSH were coincidental to the results obtained by Leoni *et al*. [20] who stated that superovulatory treatment with eCG/FSH may increase the ovarian responses compared with FSH alone. In spite of the surgical embryos collection leads to formation of post-operative adhesions that reduced the number of embryos recovered after repeated surgeries [22] but, still an easy way to optimize ovine embryos of superior genetics, endangered breed, or at the end of their reproductive lifespan. The first problem with repeated embryo recoveries after treatments with eCG is the possibility of a humoral response to this gonadotropin because its repeated injections could promote the development of anti-eCG antibodies which might affect fertility [23]. We have found non-significant difference of the oocyterian response and recovered oocytes/embryos in sheep superovulated with PMSG 1200 I.U. between the first and second superovulation that contradict the results reported by Forcada *et al*. [24]. However, Boland and Gordon [25] suggested that refractoriness of the oocyterian activity or damage and adhesions from surgical recovery might have detrimental factors on the ovulatory rate when this surgery is repeated at short intervals.

The high concentrations of anti-eCG antibodies just before another exposure to the gonadotropin are associated with a lack of or delay in the pre-ovulatory LH surge which impaired fertility after artificial insemination. This effect is much less pronounced after natural mating. Therefore, gonadotropin-releasing hormone (GnRH) after FSH treatment could be advantageous by improving synchrony of ovulations (Ovsynch) and so producing more embryos [26, 27].

The use of GnRH 24 h after sponge removal improved the fertilization rate and also the number of transferable embryos. These results were similar to those obtained by others [19, 26] probably as a consequence of a greater synchrony in the ovulation that allowed a better fertilization rate and embryo production. However, the use of GnRH wasn't adopted by the majority of practitioners [14,28]. Nancarrow *et al*. [29] reported that, ewes treated with sponge plus eCG and inseminated intrauterine with fresh semen, 75 % of the oocytes were fertilized when using GnRH compared to 49 % without GnRH.

Conclusion

Our study presented two different protocols for multiple ovulations and embryo recovery in sheep in two different habitats. The recovery rate of the harvested morulae and blastocysts in relation to the developed corpora lutea were 38.2 and 24.4 %, respectively, in the technique I. They were 40.8 and 25.8 %, respectively, in the technique II. Moreover, the percent of unfertilized oocytes and lost recovered structures.

Conflict of interest

None of the authors have any conflict of interest.

Acknowledgment

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References

[1] Bedier, N.Z.; Younis, A.A.; Galal, E.S.E. and Mokhtar, M.M. (1992): Optimum ewe size in desert Barki sheep. Small Rumin Res, 7(1): 1-7.

[2] Snowder, G.D. and Duckett, S. K. (2003): Evaluation of the South African Dorper as a terminal sire breed for growth, carcass, and palatability characteristics. J Anim Sci, 81(2): 368-375.

[3] Terawaki, Y. and Asada, Y. (2002): Relationships between distribution of number of transferable embryos and inbreeding coefficient in a MOET dairy cattle population. Asian-Aust J Anim Sci, 15(12):1686-1689.

[4] O'Callaghan, D.; Yaakub, H.; Hyttel, P.; Spicer, L.J. and Boland, M.P. (2000): Effect of nutrition and superovulation on oocyte morphology, follicular fluid composition and systemic hormone concentrations in ewes. J Reprod Fertil, 118(2): 303-313.

[5] Pintado, B.; Gutierrez-Adan, A. and Perez L. (1998): Superovulatory response of Murciana goats to treatments based on PMSG/Anti-PMSG or combined FSH/PMSG administration. Theriogenol, 50(3): 357-364.

[6] Mutiga, E.R. and Baker A.A. (1984): Effect of reduced daylight length on oestrus occurrence and superovulatory response in ewes treated with follicle stimulating hormone during the nonbreeding season. Vet Rec, 114(1): 13-15.

[7] Gonzalez-Bulnes, A.; Santiago-Moreno, J.; Cocero, M. J. and Lopez-Sebastian, A. (2000): Effects of FSH commercial preparation and follicular status on follicular growth and superovulatory response in Spanish Merino ewes. Theriogenol, 54(7): 1055-1064.

[8] Al-Kamali, A.A.; Boland, M.P.; Crosby, T.F. and Gordon, I. (1985): Reduced superovulatory response in the ewe following repeated gonadotrophin treatment. Vet Rec, 116(7): 180-181.

[9] Roy, F.; Combes, B.; Vaiman, D.; Cuibiu, E.P.; Pobel, T.; Deletang, F.; Combarnous, Y.; Guillou, F. and Maurel, M.C. (1999): Humoral immune response to equine chorionic gonadotropin in ewes: Association with major histocompatibility complex and interference with subsequent fertility. Biol Reprod, 61(1): 209-218.

[10] Smith, C.L. and Murphy, C.A. (1987): An ante grade surgical uterine flush technique for oocytes collection in the ewe. Am J Vet Res, 48:1129..

[11] Lindner, G. M. and Wright, R. W. (1983): Bovine embryo morphology and evaluation. Theriogenol, 20(4): 407-416.

[12] Leibo, S.P. (1984): A one-step method for direct nonsurgical transfer of frozen-thawed bovine embryos. Theriogenol, 21(5): 767-790.

[13] Youngs, R.C. (2011): Cryopreservation of preimplantation embryos of cattle, sheep, and goats. J Visual Exp, 54: 1-4

[14] Bari, F.; Khalid, M.; Haresign, W.; Murray, A. and Merrell, B. (2000): Effect of mating system, flushing procedure, progesterone dose and donor ewe age on the yield and quality of embryos within a MOET program in sheep. Theriogenol, 53(3): 727-742.

[15] Torres, S.; Cognie, Y. and Colas, G. (1987): Transfer of superovulated sheep embryos obtained with different FSH-p. Theriogenol, 27(2): 407–419.

[16] Abdullah, R.B.; Rahman, A.N.M.A.; Wan Khdijah, W.E. and Omar, F.N. (2001): Recent advances in goat reproduction biotechnology in Malaysia. Malays Appl Biol, 40:1-8.

[17] Espinosa-Marquez, M.C.; Valencia, J.; Escobar-Medina, F.J.; Colina-Flores, F. and Arechiga-Flores, C.F. (2004): Effect

of fluorogestone acetate on embryo recovery and quality in eCG-superovulated goats with premature luteal regression. Theriogenol, 62(3): 624-630.

[18] Okada, M.; Ishida, N.; Ogiao, T.; Itagaki, R.; Ishikawa, D. and Fuki, Y. (1999): Effect of dosage of equine chorionic gonadotropin combined with a single injection of porcine follicle-Stimulating hormone for superovulation treatment in ewes. J Rep Dev,45(5): 307-313.

[19] Jabbour, H.N.; Ryan, J.P.; Evans, G. and Maxwell, W.M.C. (1996): Effects of season, GnRH administration and lupin supplementation on the oocyterian and endocrine responses of Merino ewes treated with PMSG and FSH-P to induce superovulation. ReprodFertil Dev, 3(): 699-707.

[20] Leoni, G.; Bogliolo, L.; Pintus, P.; Ledda, S. and Naitana, S. (2001): Sheep embryos derived from FSH/eCG treatment have a lower in vitro viability after vitrification than those derived from FSH treatment. Reprod Nutr Dev, 41(3): 239-246.

[21] Holtz, W.; Wang, X.; El-Gayar, M. and Knight, P.G. (2012): The effect of exogenous gonadotropins on oocytesrian function in goats actively immunized against inhibin. Theriogenol, 77(2): 253-259.

[22] Torres, S. and Sevellec, C. (1987): Repeated superovulation and surgical recovery of embryos in the ewe. Reprod Nutr Dev, 27(4): 859-863.

[23] Forcadaa, F.; Amer-Mezianeb M.A.; Abeciaa, J.A.; Maurelc, M.C.; Cebrián-Péreza, J.A.; Muiño-Blancoa, T., Asenjod, B., Vázqueza, M.I. and A. Casaoa, A. (2011): Repeated superovulation using a simplified FSH/eCG treatment for in vivo embryo production in sheep. Theriogenol, 75(4): 769-776

[24] Forcada, F.; Meziane, A.A.M.; Abecia, J.A.; Maurel, M.C.; Cebrin-Pérez, J.A.; Muio-Blanco, T.; Asenjo, B.; Vzquez, M.I. and Casao, A. (2010): Repeated superovulation using a simplified FSH/eCG treatment for in vivo embryo production in sheep. Theriogenol, 75(4): 769-776.

[25] Boland, M.P. and Gordon. I. (1982): Effect of repeated horse anterior pituitary extract treatment on ovulatory response in the ewe. Vet Rec, 111(17): 391-392.

[26] Menchaca, A.; Vilarino, M.; Pinczak, A.; Kmaid, S. and Saldana, J.M. (2009): Progesterone treatment, FSH plus eCG, GnRH administration, and Day 0 protocol for MOET programs in sheep. Theriogenol, 72(4): 477-483.

[27] Bettencourt, E.M.; Bettencourt, C.M.; Chagas, E.; Silva, J.; Ferreira, P.; Manito, C.I.; Matos, C.M.; Romao, R.J. and Rocha, A. (2008): Effect of season and gonadotrophin preparation on superovulatory response and embryo quality in Portuguese Black Merinos. Small Rumin Res, 74(1): 134-139.

[28] Gonzalez-Bulnes, A.; Garcia, G.R.M.; Santiago-M. J.; A Lopez, S.A. and Cocero, M.J. (2002): Effect of follicular status on superovulatory response in ewes is influenced by presence of corpus luteum at first FSH dose. Theriogenol, 58(8): 1607-1614.

[29] Nancarrow, C.D.; Murray, J.D.; Boland, M.P.; Sutton, R. and Hazelton, I.G. (1984): Effect of gonadotrophin releasing hormone in the production of single-cell embryos for pronuclear injection of foreign genes. In Reproduction in Sheep, pp. 286-288. Eds D. R. Lindsay & D. T. Pearce. Australian Academy of Science, Canberra.

الملخص العربي

استخدام طريقتي المحفزات التناسلية المشيمية الخيلية وهرمون التحوصل للتبويض المتعدد وتجميع الاجنة في الأغنام بواسطة التقنيات المساعدة للتكاثر

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تهدف هذه الدراسة الي إبراز الدور الحيوي للتقنيات المساعدة للتكاثر عن طريق استخدا طريقتين مختلفتين للتبويض المتعدد وتجميع الأجنة في سلالتين مختلفتين من الأغنا هما برقى ودوربر. أجريت هذه الدراسة في تجربتين منفصلتين حيث تم اجراء التجربة الأولي بكلية الطب البيطري جامعة المنوفية بمدينة السادات في الفترة من أغسطس إلي فبراير بينما الثانية تم اجراؤها في ويرابي- فيكتوريا – ملبورن باستراليا في الفترة ما بين مارس وأبريل. في التجربة الأولي (٢٠-٢١ يو) تم اعطاء الحيوانات هرمون البروجيسترون فى الأسفنج المهبلى لمدة ١٤ يو مع حقن المحفز التناسلي المستخلص من المشيمية الخيلية (eCG) عند اليو ١٣ ثم تم تجميع الأجنة تحت تأثير التخدير الموضعي بعد ٥ أيا من التلقيح عن طريق التزاوج. من الناحية الأخري اعتمدت التجربة الثانية (١٨ يو) علي اعطاء الحيوانات هرمون البروجيسترون عن طريق نظا السيدار المهبلى لمدة ١٢ يو مع حقن هرمون التحوصل (FSH) ٦ مرات على مدار ٣ أيا وحقن المحفز التناسلي المستخلص من المشيمية الخيلية (eCG) عند اليو ١٢ ثم تم تجميع الأجنة بعد ٤ أيا من التلقيح داخل الرحم بواسطة المنظار. كانت النتائج تشير الي تحسن نسبة التعافي للتوتية (Morula) والكيسية (Blastocyst) المرتبطة بالأجسا الصفراء النامية في التجربة الثانية عن الأولي بنسبة ٤٠.٨ % مقابل ٢٥.٨ % للتوتية و٣٨.٢ % مقابل ٢٤.٤ % للكيسية. بينما كانت نسبة البويضات الغير مخصبة أو التي فقدت أثناء استخلاص الاجنة عالية في التجربة الأولي مقارنة بالثانية. نستخلص من هذه الدراسة أنه على الرغم من اختلاف النتائج بين التقنيتين والتى قد تعزى الى اختلاف البيئة محل الدراسه واختلاف الأعمار الخاصة بالحيوانات فان نسبة الأجنة التى قد تم الحصول عليها من نعاج السلالاتين والتى لم تتجاوز أعمارهم ستة أعوا لم تقل عن ٣٨ % مع اختلاف التقنية المستخدمه فى كلا الدراستين.