Egyptian Journal of Sheep and Goat Sciences, Vol. 8 (1), P: 111-117, 2013
(Proceedings Book of 4th International Scientific Conference on Small Ruminant Development)
(3-7 Sept., 2012, Sharm El Shiekh)

EFFECT OF REGULATING MATING SYSTEM ON SEXING OF RAHMANI LAMBING

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SUMMARY

The experimental work was carried out as field welfare trial on Rahmani ewes to explain the effect of the interval time between proem of estrus and mating time on sexual ratio and reproductive performance. Data were obtained from 186 mature and healthy ewes without any problem in estrus and parturition. All experimental ewes were nourished biological corn silage before breeding season up to 34 days as flushing period. At the beginning of the trial, the ewes were allocated into three groups (n=62 each). The G1 (as control) was received fertile rams at instant of estrus. Both G2 and G3 were surrendered to mating at 15 hours and at 30 hours from the visual detection of estrus, respectively. Eight rams without significantly differences in testicular measurements and semen characteristics were serviced ewes flock. The present results showed that systematic mating time during estrus has been believed to influence the conception rate and gender offspring ratio. The conception rate in G1, G2 and G3 was 75.81 %, 88.71 % and 95.16 %, respectively. The sexing parturition number between males and females was 20: 19 in G1, 9: 49 in G2 and 61: 10 in G3, respectively. The sexing ratio between male and female lambs was 51.28 % and 48.72% in G1, respectively. Delaying mount was awarded to a significant deviation in sexing towards females (84.48%) in G2 and males (85.92%) in G3. It can be concluded that interval time between the initiate of estrus and mating is a reliable tool for fetal sex determination in Rahmani ewes. As conclusion, , if breeders have more attention to sexing lambs, they could arrange time of mating in flock. Mating in middle of estrus could yield more female lambs while, retard mating during estrus might give more male lambs than mating at the onset of estrus.

Keywords: *Ewes welfare, mating time system, vaginal pH, sexing lambs*, reproductive performance.

INTRODUCTION

Animal welfare is the combination of subjective and objective (qualitative and quantitative) aspects of the conditions of life for animals including: health, disease, behaviour, nutrition planning and organizing mating season. The welfare and regulation of mating system in animals through breeding season have played a main role in maintaining offspring number, gender and healthy. The welfare of estrus ewes in heating season is not easily detected when ewe has been separated from ram for a period of time. Jordan (2005) suggested that estrus can be divided into phases; the follicular phase 3-4 days, and the luteal phase that lasts about 14-15 days which characterized by the maturation of corpus luteum and high levels of progesterone. The length of estrous cycle in ewes are varied from 14-19 days (Hashemi et al., 2006). The duration of estrus time ranges between 18 and 72 hours with an average of 36 hours (Dogan and Nur, 2006). The ovulation is event at 21.9 - 38.9 hours with a mean 29.8 hours from the onset of estrus (Rodrigues et al., 2007). However, Wagenmaker et al. (2009) stated that estrus length of ewe is17 days (13-17), estrus duration 30 hours (18-48), ovulation 24-30 hours from onset of estrus and LH surge to ovulation at 26 hours. The predetermination of sexing has been a goal of livestock producers for generations. Sexing is one of the factors affect birth weight and pre-weaning growth of lambs. The development of differences in birth weight of male and female lambs had been started during pregnancy. There is an unquestionable interest in the sex ratio for evolutionary, biological and genetic reasons (Klug et al., 2010). From the economic point of view, sex ratio could be important. Some protocols have been established for isolation of spermatozoa bearing X and Y chromosomes such as centrifugation (Carvajal et al., 2004), Percoll gradients (Wolf et al., 2008), embryos transfer (Sun, 2010) and intervals mating time accuracy of fetal sexing (Rorie, 1999, Santos et al., 2007 and Khalifa et al., 2010).

Therefore, the present study was aimed to monitor the effectiveness of regulating mating time on sexing and reproductive pattern at inception estrus, middle estrus at 15 hours and late estrus at 30 hours in Rahmani ewes.

MATERIALS AND METHODS

This study was conducted in El-Serw Breeding Station, Domietta Governorate belonging to Animal Production Research Institute, Egypt. The experimental work was carried out during breeding season up to parturition.

Feeding and management of ewes

A total of 186 mature and clinically healthy, fertile, and in estrus Rahmani ewes were used in this study. The body condition score (BCS, ranging from 1 to 5) were evaluated by palpation of the lumbar and sternal region on day of estrus. The specifications of ewes as BCS ≥ 2.9, aging ≥ 2.3 years and live body weight ≥ 35.13 kg at start of the trial. The ewes were received commercial concentrate feed mixture (CFM) and biological treated corn silage before mating for 34 days as care period. When breeding season began, ewes were kept on collective pens and fed CFM and silage twice a day to cover the production requirements. The feed requirements before and during mating season were adjusted within NRC (2007). The CFM contained: yellow maize (39%), undecorticated cottonseed meal (25%), wheat bran (20%), soybean meal (7%), molasses (5%), limestone (2.5%), common salt (1%) and minerals mixture (0.5%). The fresh water and mineral salt blocks were permanently available. Composition and analysis of feedstuffs were applied according to AOAC (1995) and presented in Table 1.

Table 1: The composition of the experimental feedstuffs.

Components(%)	Feedstuffs	
	CFM	Biological silage
Organic matter	93.30	90.72
Crude fiber	14.91	26.76
Crude protein	15.24	10.86
Ether extract	3.42	3.35
Nitrogen free extract	59.73	49.75
Ash	6.70	9.28

Estrus detection and mating regulation system

Estrus detection was applied 8 times during the 24 hours (two hours to discover heat and one hour as rest period) at mating season. Ewes experiencing estrus behavior will stand to be mounted by other ewes or detector ram. Characteristic behavior for estrus ewe is nervousness, walking near the fence or increasing vocalizations for the ram. Physical characteristics include a reddened and swollen vulva. The ewes came in heat were serviced once within three scheduled natural mating times. Group one (G1) was used as control, serviced at onset of estrus (0 time). Either group two (G2) or three (G3) received ram at 15 and 30 hours after onset of estrus, respectively. The vaginal pH at the three mating times (0, 15 and 30 h) was recorded according to (Khalifa et al., 2010).

Determination of pregnancy and reproductive performance

After mating season, ewes that did not displayed heat up to 42 days were considered pregnant. The reproductive performance of experimental groups included; the pregnancy rate which calculated as number of conceived ewes/ number of mated ewes, ewes lambing rate which calculated as number of ewes lambing / number of ewes conceived, single rate which calculated as the number of ewes lambing single/ total number of ewes lambed, twin rate which calculated as number of ewes lambing twin / total number of ewes lambed and triplet rate which calculated as number of ewes lambing triplet / total number of ewes lambedand sexing lambs % (male:female) which equal No. of born lambs in particular sex/Total No. of born lambs.

Feeding and management of vaccinated rams

During the breeding season, herd rams aged ≥ 2.6 years, weighed ≥ 59 kg, housed separately and received similar ration. Semen ejaculation and testicular measurements were estimated before breeding season to eliminate differences in semen and testicular size characteristics. Finally, semen ejaculates were collected from identical eight rams by a warm artificial vagina (n=12 ejaculates / ram). The ejaculates were immediately brought to the laboratory and placed in a water bath at 37°C. The semen volume was measured directly using a graduated collection tube with 0.1 ml intervals. Seminal pH was measured immediately using a digital pH meter. The progressive motile sperm were determined microscopically (×400) by placing a drop of diluted semen (1:20) on a warm slide (35°C) and observing 300 motile sperm. The live and abnormal spermatozoa were determined by observing 300 spermatozoa, after eosin-nigrosin staining using a light microscope at a magnification of × 400. Sperm density was determined by Neubaur hemocytameter, diluting a small drop of the semen with 2% eosin solution (1:200). Testicular measurements as scrotal circumference by using a tape measure and the length and width were determined using a caliper. The statistical analysis of seminal characteristics and testicular measurements indicated non-significant difference as shown in Table 2.

Statistical analysis

Data were statistically analyzed using the general linear model (GLM) procedure of SAS (2009). Differences among the means were considered significant at $P<0.05$. The differences amongst experimental groups in sexing lambs were statistically analyzed by X^2.

Table 2: Means of ram's testicular measurements and seminal characteristics in breeding season.

Measurements	Experimental rams							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Scrotal circumference (cm)	30.5 ± 1.5	30.2 ± 1.5	32.1 ± 1.3	29.5 ± 1.1	31.7 ± 1.5	29.7 ± 1.2	32.4 ± 1.1	32.2 ± 1.2
Testis width (cm)	11.7 ± 1.3	11.5 ± 1.4	12.3 ± 1.6	11.4 ± 1.2	11.6 ± 1.3	11.5 ± 1.3	12.1 ± 1.4	12.2 ± 1.7
Testis length (cm)	12.7 ± 0.3	12.6 ± 0.4	12.8 ± 0.2	12.4 ± 0.3	12.6 ± 0.4	12.5 ± 0.5	12.7 ± 0.1	12.6 ± 0.3
Seminal volume (ml)	1.13 ± 0.1	1.11 ± 0.1	1.32 ± 0.1	1.10 ± 0.1	1.21 ± 0.1	1.12 ± 0.1	1.31 ± 0.2	1.33 ± 0.1
Seminal pH	6.70 ± 0.0	6.71 ± 0.1	6.71 ± 0.1	6.71 ± 0.0	6.72 ± 0.1	6.69 ± 0.0	6.72 ± 0.1	6.70 ± 0.0
Sperm motility (%)	85.35 ±6.1	85.11 ±6.3	85.65 ±5.5	85.24 ±6.2	85.45 ±5.4	85.35 ±6.0	85.67 ±5.7	85.69 ±5.8
Live sperm (%)	87.65 ±8.1	88.75 ±7.5	90.45 ±8.4	86.89 ±7.6	89.85 ±7.2	86.67 ±7.5	90.52 ±8.5	90.67 ±8.3
Abnormal sperm (%)	11.31 ±2.2	11.42 ±2.4	11.12 ±1.6	12.30 ±2.5	12.01 ±2.1	12.11 ±2.3	11.21 ±1.7	11.42 ±1.5
Sperm concentration ($nx10^9$)	4.14 ± 0.4	4.08 ± 0.3	4.01 ± 0.3	4.14± 0.3	4.13 ± 0.3	3.99 ± 0.2	4.11 ± 0.3	4.12 ± 0.3

Means in the same row indicate non- significant difference.

RESULTS AND DISCUSSION

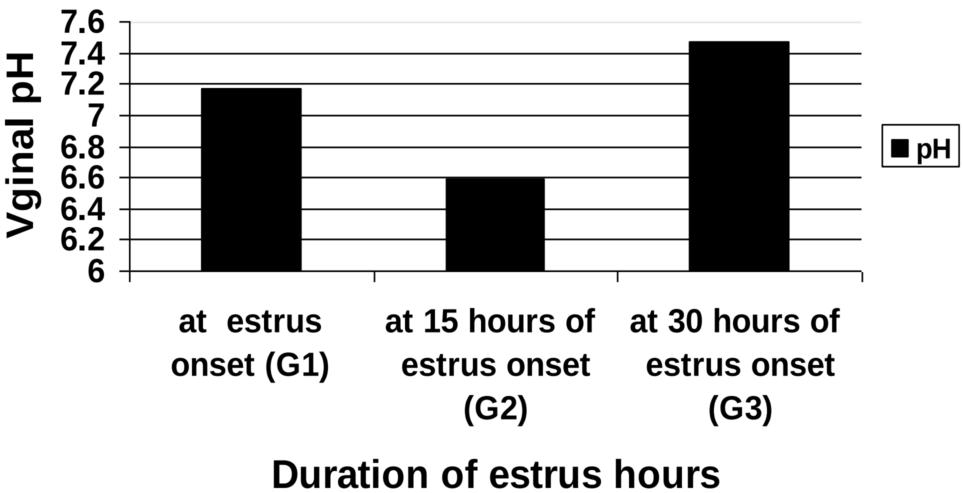
Vaginal pH

Figure 1 summarized the vaginal pH at mating time. The vaginal pH in G1, G2 and G3 at mating hour was 7.17 ± 0.53, 6.59 ± 0.23 and 7.47±0.44, respectively. The present data show significant difference ($P<0.05$) among ewe groups in vaginal pH at mount. Changing of vaginal pH (acidity) was occurred at 15 hours (G2) while, alkalinity recorded at estrus onset (G1) and 30 hours (G3). Differences between genital pH and sperm swimming data reported by Diasio and Glass (1971) reported that vaginal pH affects differently on X and Y-chromosomes bearing sperm. An acidic vaginal environment reduced dash of Y sperm, contrariwise X sperm swimming fast behind to fertilize the ovum. Reduction of vaginal pH depends upon an influx of ions such as hydrogen, sodium and chloride into the vaginal. The present results regarding vaginal pH changes are in agreement with Suárez et al. (2006) who suggested that during estrus the vaginal pH tended to be alkaline during follicular stage (early estrus), posteriorly become acidic in non-follicular stage (middle estrus) and at growing follicular stage (lutal phase) it changes towards alkaline. Also, Yesilmen et al. (2008) reported that vaginal pH was affected by interaction between hormone, microorganism and discharge of mucus caused by base of vaginal pH. The acidic and alkaline vaginal were available if the ion H^+ and ion OH^- were excess, respectively. Accordingly, Khalifa et al. (2010) found that the sperm charge (X^- and Y^+) and vagina charge (H^+ and OH^-) differences played an important role to control swimming sperm by affecting the attraction power.

Reproductive performance

Table 3 explains that reproductive performance of G1, G2 and G3 ewes responded to regulating mating time during estrus. The results indicated significant flexibility ($P<0.05$) in mating system structure within experimental ewe groups. The mammalian males produce semen in which 50% of sperm carry X chromosome and 50% carry Y chromosome. Many theoretical differences between the male and female producing sperm have been included physical differences such as size, weight, density, swimming speed, electrical surface charge, surface macromolecular proteins and differential effects of pH (Garner and Seidel, 2008).

Regarding pregnancy rate in this study of G2 and G3 were transcended compared to G1. It was 75.81% in G1, 88.71% in G2 and 95.16% in G3. This supremacy trend may be attributed to delay mating system. These results are supported by Hare and Bryant (1985) who reported that pregnancy rate was lowered up to 20% in ewe mated at onset of estrus than ewes mated at delay time of estrus. Moreover, Rondeau and Sainte-Marie (2001) suggested that the structure of mating system can have a profound effect on sexual selection strength and maintenance of secondary sexual characteristics. The systematic mount is required for ovarian follicles to become competent to undergo fertilization and further



Figer 1: Vaginal pH at mating time in the three experimental ewe groups.

development as competence is only achieved around the time of the ovulatory LH surge (Mitchell et al., 2002). Ovarian follicular development is non cease process and successive waves of follicles continue to develop throughout anestrous (Garcia et al., 2004). On the other hand, Khalifa et al. (2010) concluded that arrangement of insemination time at 0, 12, 24 and 36 hours during estrus phase realized kidding rate (%) 54.55, 81.82, 100 and 100 in goats, respectively.

Concerning litter size in the present study, postponement of mating time inspired higher ($P<0.05$) litter size in G2 and G3 than G1 (Table 3). The best litter size at 15 and 30 hours may be attributed to improvement, increasing and growth of the mature egg presented in fertility place in genital tract. Deligiannis et al. (2005) decided that litter size in ewes was related to increase number of the total follicles population within inseminating time lateness. Differences in pregnancy rate and litter size may be due to substantial factors such as timing of ovulation or the structure of cervix at early estrus. Mostly delay mating yielded acceptable conception rates and litter size. The present results are in agreement with Sukanya (2009) who recorded that adjustment of mating time is the most important factor in achieving high conception rate and good litter because sperm cells have to be in the vicinity and ready to fertilize the eggs upon ovulation.

In relation to sexing rate, the obtained male and female lambs show different ($P<0.05$) results among trial groups (Table 3). Both X and Y chromosome-bearing sperms are different morphologically and structurally. The X chromosome-bearing sperms are 2 or 3 folds greater in size than Y chromosome-bearing sperm. The X-bearing sperm has 2.8% more DNA than the Y-bearing sperm. X sperm swims more slowly and has a longer life span (Paasch et al., 2007). Furthermore, Schulman and Karabinus (2005) suggested that environmental factors like reproductive secretions including follicular, tubal and vaginal fluid pH can affect the sex ratio and X and Y chromosome population. Moreover, Hewitt (1998) observed that acid condition decrease the loss of potassium from Y- spermatozoa that may cause physiological significance and maintain intracellular sperm from loss of swimming. There are some factors such as ATP and pH associate with sperm motility. The genital pH acidity (increasing ion H^+) and negative X-sperm charge may activate speed of X-bearing sperm to reach fertilizing side earlier than Y-bearing sperm. These results are defined by Satoru et al. (1984) who found that mobility of X-bearing sperm was faster than Y-bearing sperm in acidic medium because X-bearing sperm have a higher net negative charge on the sperm cell surface than Y-bearing sperm. Also the authors indicated that female sperm has a longer life span in acidic environment than the Y sperm, though exhibit decreased motility. Moreover, the H^+ ion plays an important role in mitochondrial mid-piece region which change voltage to produce ATP energy source for spermatozoa. Holm and Wishart (1998) found that increase in Y sperm velocity and the percentage of motile sperm at alkaline pH (increasing ion OH^-) has impact on sperm male motility. It is possible that X and Y chromosomes have many physiological differences due to difference in motility, time of capacitation and survival time in reproductive tract. Shuster (2009) recorded that operational sex ratio is an important ecological factor that can influence mating system structure. The pH level in the vagina and cervix can have an effect on gender of lambs. The X sperm was more robust and slower than Y sperm and resilient to acidity in the vagina. Despite, X sperm may be slower but, it may be able to withstand an acidic environment that weed out the Y-chromosome sperm. Alkalinity would tend to favor the alacrity of male sperm or at least even the pH odds. Khalifa et al. (2009) attained that extender with acidic media (pH 6.6) activated X sperm to give 66.67 %, females offspring, while alkaline media (7.3) realized 81.25 % male lambs. Therefore, as semen deposition at genital tract might take place early or late, thus provide probability of female or male. Similarly, Khalifa et al. (2010) achieved that percentage of female and male kids were 84.62% and 73.08% when goats inseminated early at 24 h or late (36 hours), respectively.

Table 3: Reproductive performed with natural mating in Rahmani ewe groups.

Items	Natural mating with ewe groups		
	G1	G2	G3
Mating ewes	62	62	62
Conceived ewes	47[b]	55[a]	59[a]
Pregnancy rate %	75.81[b]	88.71[a]	95.16[a]
Lambing ewes	37[b]	47[a]	50[a]
Lambing ewes %	78.72[b]	85.45[a]	84.75[a]
Total number of lambs	39[c]	58[b]	71[a]
No. of ewes lambed Single	35	36	31
Single rate %	94.59[a]	76.60[b]	62.00[c]
No. of ewes lambed twin	2	11	17
Twinning rate %	5.41[c]	23.40[b]	34.00[a]
No. of ewes lambed triplet	-	-	2
Triplet rats %	-	-	4.00
No. of males	20	9	61
Males rate %	51.28[b]	15.52[c]	85.92[a]
No. of females	19	49	10
Females rate%	48.72[b]	84.48[a]	14.08[c]

Values with different superscripts in the same row, differ significantly (P<0.05).

CONCLUSION

Based on the results presented herein, a significant association was evident between regulating mating time and lambs' sexing. The time of mating during estrus has been recognized to influence the sex ratio of offspring. Middle mating at 15 hours could achieve more females (84.48%) while late mating at 30 hours could attain more males (85.92%). In addition, sexing offers clear opportunity to increase efficiency of production through modifying reproductive performance.

REFERENCES

A.O.A.C. (1995). Official Methods of Analysis (16th) Edt. Association of Official Analytical Chemists, Washington, D.C., USA.

Carvajal, G., Cuello, C., Ruiz, M., Vazquez, J. M., Martinez, E. A. and Roca, J. (2004). Effects of centrifugation before freezing on boar sperm cryosurvival. J Androl. 3:389–396.

Deligiannis, C., Valasi, I., Rekkas, C. A. P., Theodosiadou, E., Lainas, T. and Amiridis, C. S. (2005). Synchronization at ovulation and fixed time intrauterine insemination in ewes. Reprod. Dom. Anim., 40: 258-272.

Diasio, R. B. and Glass, R. H. (1971). Effects of pH on the migration of X and Y sperm. Fertil. Steril., 22 (5): 303-305.

Dogan. I. and Nur, Z. (2006). Different estrous induction methods during the non-breeding season in Kivircik ewes. Veterinarni Medicina, 51(4): 133–138.

Garcia, R. M., Inskeep, E. K., Lopez-Sebastian, A., Mcneilly, A. S., Santiago - Moreno, J., Souza, C. J. H. and 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.

Garner, D. L. and Seidel, G. E. (2008). History of commercializing sexed semen for cattle. Theriogenology. 69: 886–895.

Hare, L., and Bryant, M. J. (1985). Ovulation rate and embryo survival in young ewes mated either at puberty or at the second or third oestrus. Anim. Reprod. Sci., 8:41.

Hashemi, M., Safdarian, M. and Kafi, M. (2006). Estrous response to synchronization of estrus using different progesterone treatments outside the natural breeding season in ewes. Small Rumin. Res., 65: 279-283.

Hewitt, J. (1998). Preconceptional Sex Selection. Br. J. Hosp. Med., 37:149, 151-2, 154-5.

Holm, L. and Wishart, G. J. (1998). The effect of pH on the motility of spermatozoa from chicken, turkey and quail. Anim. Reprod. Sci., 54: 45-54.

Hunter, R. H. F., Nichol, R. and Crabtree, S. M. (1980). Transport of spermatozoa in the ewe: timing of the establishment of a functional population in the oviduct. Reprod. Nutr. Dévelop., 20:1869-1875.

Jordan, K. M. (2005). Approaches to improve the ovulatory response and reproductive performance of ewes introduced to rams during seasonal anestrus. West Virginia: West Virginia University. P. 90.

Khalifa , E .I., Ahmed, M. E., Abdel- Gawad, A. M and El-Zelaky, O. A. (2010). The effect of insemination timing on fertilization and embryo gender in zaraibi goats. Egypt. J. of Sheep & Goat Sci., 5 (1): 271-281.

Khalifa, E. I., Ahmed, M. E., Abdel-Salaam, I., El-Zelaky, O. A and Abdel-Gawad, A. M. (2009). Effect of semen extender pH value on Rahmany ram characteristics and on altering sex ratio of offspring. Agric., Research J., Suez Canal Univ., 9 (1): 49-52.

Klug, H., Heuschele, J., Jennions, M. D. and Kokko, H. (2010). The measurement of sexual selection. J. of Evolutionary Biology. 23:447–462.

Mitchell, L. M., Dingwall, W. S., Mylne, M. J. A. and McEvoy, T. G. (2002). Season affects characteristics of the pre-ovulatory LH surge and embryo viability in superovulated ewes. Anim. Reprod. Sci., 74:163-174.

NRC. (2007). Nutrient requirements of small ruminants: sheep, goats, cervids, and New World camelids. National Research Council of the National Academies, National Academies Press, Washington, D.C., U.S.A.

Paasch, U., Grunewald, S. and Glander, H. J. (2007). Sperm selection in assisted reproductive techniques. Reprod. Fertil., 65: 515-525.

Rodrigues, P. A., Coelho. L. A., Nonaka, K. O., Sasa, A., Vicente, W. R. R., Balieiro, J. C. C. and Siqueira, E. R. (2007). Annual characteristics of estrous activity in wool and hair ewe lambs under subtropical conditions. Sci. Agric. (Piracicaba, Braz.). 64 (5): 468-475.

Rondeau, A. and Sainte-Marie, B. (2001). Variable mate-guarding time and sperm allocation by male snow crabs (*Chionecetes opilio*) in response to sexual competition, and their impact on the mating success of females. Biological Bulletin 201:204–217.

Rorie, R. W. (1999). Effect of timing of artificial insemination on sex ratio. Theriogenology. 52 (8): 1273-1280.

SAS. (2009). SAS/STAT® 9.2 User's Guide, 2nd edn. SAS Institute Inc, Cary, N.C., U.S.A.

Satoru, K., Shigeru, O., Toshifumi, K. and Rihachi, I. H. M. (1984). Human X- and Y-bearing sperm differ in cell surface sialic acid content. Biochemical and Biophysical Research Communications. 124 (3): 950-955.

Santos, M. H. B., Gonzalez, C. I. M., Bezerra, F. Q. G., Neves, J. P., Reichenbach, H. D., Lima, P. F. and Oliveira, M. A. L.(2007). Sexing of Dorper sheep fetuses derived from natural mating and embryo transfer by ultrasonography. Reprod. Fertil. and Develop., 19(2):366–369.

Shuster, S. M. (2009). Sexual selection and mating systems. Proceedings of the National Academy of Sciences of the USA 106 (suppl.):10009–10016.

Suárez, G., Zunino, P., Carol, H. and Ungerfeld, R. (2006). Changes in the aerobic vaginal bacterial mucous load and assessment of the susceptibility to antibiotics after treatment with intravaginal sponges in anestrus ewes. Small Rumin. Res., 63: 39–43.

Sukanya, L. (2009). Development of trans-cervical artificial insemination in sheep with special reference to anatomy of cervix. Suranaree J. Sci., Technol., 17(1):57-69.

Sun, S. (2010). Effects of environment on gestation ratio of sheep embryo transfer and its control measures. Journal of Agricultural Science. 2(2):234-238.

Wagenmaker, E. R., Breen, K. M., Oakley, A. E., Pierce, B. N., Tilbrook, A. J., Turner, A. I. and Karsch, F. J. (2009). Cortisol interferes with the estradiol-induced surge of luteinizing hormone in the ewe. Biol. Reprod., 80:458–463.

Wolf, C.A., Brass, K.E., Rubin, M.I.B., Pozzobon, S.E.,. Mozzaquatro, F. D. and De La Corte, F.D. (2008). The effect of sperm selection by Percoll or swim-up on the sex ratio of *in vitro* produced bovine embryos. Anim. Reprod., 5 (3-4):110-115.

Yesilmen, S., Ozyurtlu, N., Kucukaslan, I. and Altan, F. (2008). The effect of progestagen on the vaginal flora arising from intravaginal sponge treatment and susceptibility of the vaginal florato antibiotics in ewes. J. Anim. Vet. Adv., 7: 1418–1421.