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Full Length Research Paper

Sex ratio of lambs born from assisted reproductive technologies

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The objective of the present study was to investigate sex ratio of offspring resulting from superstimulated donors from two different breeds and artificially inseminated ewes from three different breeds. Romanov (n = 5) and Charollais (n = 6) (2 - 7 years of age) donors were superovulated using FSH-p with 400 mg NIH-FSH-P1 (total of 20 mg). Six days embryos were recovered surgically and grade 1 embryos (IETS classification) were frozen **in** ethylene glycol**. Embryos (n = 154) were transferred into recipient ewes in pairs. A total 267 fat tailed ewes were synchronized (60 mg MAP and 600 I.U. PMSG) and inseminated with fresh diluted semen (10 x 10^7). Percentage for female sex ratio (64%) of offspring born from frozen thawed embryos differed significantly (P < 0.05) than expected ratio of 50:50. Lambing rates for recipient carried Romanov embryos (73.8%) was significantly higher (P < 0.05) than those carried Charollais embryos (57.1%). Pregnancy rates of artificially inseminated ewes were differed (P < 0.05) at time of insemination. Sex ratios of offspring were found similar among ewes with different breeds, inseminated in different times and with different vaginal mucus electric resistance. In summary, there is an advantage in the sex ratio obtained when using superstimulated ewes.**

Key words: Sex ratio, assisted reproductive technologies, sheep.

INTRODUCTION

Assisted reproductive technologies, including artificial insemination (AI) and embryo transfer (ET) have developed into powerful reproductive flock management techniques. Currently, AI alone, or in combination with an ET program, allows producers access to genetics they would normally be unable to obtain or afford to acquire. Timon and Hanrahan (1985) indicated that implementing an embryo transfer program in addition to AI would yield substantial genetic improvement. Most economically important genetic traits can be manipulated with relative ease (Land and Hill, 1975), while sex selection has, until recently, remained a matter of chance. Recent technological advances have allowed for the development of sex selection methods that can increase the probability of producing offspring of the desired sex. However, due to the relative high cost and limited efficiencies, the potential benefits of utilizing sex selecting techniques would most likely be realized in programs where the procedural cost would not outweigh the ability to disseminate the elite genetic potential (Seidel, 2003). The development of simpler and relatively inexpensive sex selection techniques will increase the use of assisted reproductive technologies, such as AI and ET, in production animal agriculture. Thus we investigated if there is a deviation of expected sex ratio of lambs born from ART.

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MATERIAL AND METHODS

The timing of oestrus was synchronized in donors and recipients with the placement of intravaginal sponges (Veramix, Upjohn, Orangeville, Ontario, Canada) containing 60 mg medroxyprogesterone acetate for 14 days. Recipients were injected with 400 IU eCG (Equinex, Ayerst, St Laurent, Quebec, Canada) at sponge removal. Romanov (n = 8) and Charollais (n = 7) (2 - 7 years of age) donors were superovulated using FSH-p with 200 mg NIH-FSH-P1 (total of 20 ml) (Folltropin-V; Vetrepharm, Canada) applied in 8 decreasing doses of 1.5, 1.5, 1.5, 1.25, 1.25, 1, 1, 1 ml i.m. at 12 h intervals, starting 60 h before sponge withdrawal. Donors underwent intrauterine insemination with fresh diluted semen (10 x 10^7) 40 h after sponge removal. Six days embryos were recovered surgically from the day of artificial insemination and grade 1 embryos (IETS classification) were frozen in ethylene glycol. Straws were thawed by holding in air for 10 s and then transfer into 35°C water for 30 s.

Table 1. Mean (±S.E.M.) number of transferable embryos from superovulated ewes, pregnancy rates and sex ratio of offspring.

Donor genotype	n	No. of transferable embryos	Pregnancy rates (%)	Embriyo survival rates (%)	Female sex ratio (%)
Romanov	8	10.6 ± 1.81	73.8 ± 10.75[a]	72.8 ± 7.18	65 (30/46)*
Charollais	7	9.9 ± 1.94	57.1 ± 11.49[b]	70.3 ± 6.68	64 (18/28)

[ab]Data bearing a different superscript were significantly at P < 0.05.
*Significantly different than expected ratio 50:50 at P < 0.05.

The embryos were recovered from the straw and transferred into holding media for 5 min to equilibrate and reexpand before transferring to recipients. A total of 154 thawed embryos were transferred into recipient ewes (n = 77) in pairs.

A total of 267 three different breed of fat tailed ewes with different mature weight (Kangal = 60 – 65 kg, n = 117; Akkaraman = 40 – 45 kg, n = 69 and Morkarman = 50 - 55 kg, n = 81) were used. Vasectomized rams were introduced to better synchronize ewes and to mark ewes as they came into heat at the rate of 5 rams per 100 ewes. The animals were screened for estrus beginning at 24 h after sponge removal and continuing up to 57 h. Screening was performed every day between 9:00 am and 9:00 pm. Animals that did not show any mating marks by 57 h were not inseminated. Marked ewes received i.v. injection of an anesthetic cocktail containing 2cc Ketasol (Indus Pharma, Karachi, Pakistan) + 0.04cc Romphun (Bayer) and were inseminated 52 to 57 h after sponge removal with fresh diluted semen containing 100 x 10^6 motile spermatozoa. Electric resistances of vaginal secretions were measured with probe (DRAMINSKI, Poland) gently inserted to vagina just prior to insemination. One experienced laparoscopic artificial insemination operator performed the inseminations. Semen was then deposited into the lumen of the mid portion of each uterine horn. Two months after the insemination, conception rates of animals of all groups were checked by transabdomial ultrasonography, using B-mode diagnostic ultrasound scanner (100 Falco, pie medical application manual, equipment B.V., Maastricht, Netherland).

Statistical analyses were conducted using GENSTAT (Version 8.1, VSN international, Hemel Hempstead, UK). Pregnancy data were analyzed by binomial logistic regression, whereas the mean number of embryos transferred was analyzed by general analysis of variance. Deviations from a 1:1 sex ratio were tested by binomial tests.

RESULTS

The mean number of grade 1 embryos (those classified as late morulae, early blastocysts, blastocysts, expanding blastocysts) was similar between Romanov and Charollais breeds. The percentage of embryos transferred that resulted in a fetus at ultrasound pregnancy (55d) diagnosis was higher (P < 0.05) in recipient carried Romanov embryos than those carried Charollais. The proportional female sex ratio in both breed of lambs born from frozen embryo transfer was significantly higher than the expected sex ratio 50:50 (P < 0.05) (Table 1).

The time of insemination after the sponge withdrawal had effect on pregnancy rates (p < 0.05). The pregnancy rate at 55 d of gestation in ewes inseminated later (56 - 57 h) was higher than those inseminated earlier (52 - 55 h). Neither breed of sheep nor electrical resistance of vaginal secretion at the time of insemination affected pregnancy rates. Surprisingly litter size differed (P < 0.05) according to electrical resistance of vaginal secretion which is correlated with maternal hormone levels. Even though estrogen concentrations are not validated with vaginal electrical resistance (VER) values in the current study, based on the previous researches (Szczepanski et al., 1994; Bartlewski et al., 1999; Rezac, 2008) ewes with lower VER, which means higher estrogen levels, produced more offspring. Similarly when VER values is minimum which means estrogen pikes, ewes conceived to more (P > 0.05) males than females. The percentages of female lambs resulting from the early and late inseminations were 45 and 54.8%, respectively. There was a slight, nonsignificant male-bias among lambs born to ewe breeds weighing more than other two breed of ewes. In particular, larger size of sheep breed (Kangal) leaded to the birth of not significantly but relatively more male (60.4%) than female offspring. The secondary sex ratio at birth in medium size Morkaraman and small size Akkaraman were found similar to the theoretical 1:1 expected ratio (Table 2).

DISCUSSION

It is desirable to control the sex of offspring for the livestock industry. In many species, early insemination or mating appears to favor female offspring, whereas late mating appears to favor male offspring (Gutierrez-Adan et al., 1999). Artificial insemination is typically delayed until late estrus or after the end of estrus whereas natural mating must occur during estrus. If the time of insemination influences the sex ratio of offspring, then it might be expected that artificial insemination would result in a higher percentage of male offspring. In the current study for inseminations held within 55 h of the sponge removal, 45% (28 of 62) of the lambs produced were females. Contrary to the previous study (Gutierrez-Adan et al., 1999) timing of insemination had no effect on the sex of lambs born.

Several recent studies (Wehner et al., 1997) have used methods to time insemination more precisely in relation to ovulation. It was reported that resistance of vaginal secretions is inverse to estrogen concentration (Rezac, 2008) and, thus, is expected to reach minimum values

Table 2. Mean (±S.E.M.) number litter size, pregnancy rates and sex ratio of offspring born from artificially inseminated ewes.

Parameter	n	Pregnancy rate (%)	Litter size	Female sex ratio
Breed of Sheep				
Akkaraman	69	37.7 ± 5.9	1.2 ± 0.09	54.0 (20/37)
Kangal	117	36.8 ± 4.5	1.3 ± 0.07	39.6 (19/48)
Morkaraman	81	47.0 ± 5.4	1.2 ± 0.07	50.0 (23/46)
Time of Insemination				
52 - 53 h	45	33.3 ± 7.2[a]	1.2 ± 0.10	45.5(10/22)
54 - 55 h	122	35.3 ± 4.4[a]	1.2 ± 0.07	45.0(18/40)
56 - 57 h	100	49.0 ± 4.8[b]	1.3 ± 0.06	54.8 (34/62)
Electrical resistance of vaginal secration				
18 - 23	62	38.7 ± 6.3	1.4 ± 0.09[a]	40.6 (13/32)
24 - 29	53	39.6 ± 6.8	1.4 ± 0.10[a]	57.7 (15/26)
>30	152	40.8 ± 3.9	1.1 ± 0.05[b]	51.5 (34/66)

[ab]Data bearing a different superscript were significantly at $P < 0.05$.

when estrogen peaks at estrus. As estrogen declines after the gonadotrophin surge, resistance of secretions increases again. By measuring and plotting changes in the resistance of vaginal and cervical secretions it is possible to estimate the time of onset of estrus and the gonadotrophin surge (Rorie, 1999). In the current study, we inseminated ewes in estrus when resistance was between 18 and >30, minimum probe readings (18 - 23) resulted in 19 of 32 (59.4%) male lambs. Thus we speculated that the inseminations at high estrogen concentrations successfully altered the sex ratio because Y bearing sperm would be more likely to fertilize the oocyte. This finding agreed with James (1990) who reported that male births have been closely correlated with high estrogen concentrations at the time of conception in human. Canfield and Butler (1989) reported that the lowest re-sistance value was within +6 h of the LH surge in 79% of the animals and l2 h of the LH surge in 89% of the animals. In our study, difference in the litter size according to vaginal resistance indicated that ewes with higher resistance value were inseminated at ovulatory mistiming. Premature ovulations (Driancourt and Fry, 1992), ovulatory delays and ovulatory asynchronies (Cognie et al., 2003) have been described with these mistimed ovulations causing subsequent fertilization failures, due to the presence of aged oocytes (Takahashi et al., 2003) and/or aged spermatozoa (Jabbour and Evans, 1991) at the time of fertilization.

Maternal skewing of offspring sex ratio might have important agricultural implications. Offspring of one gender may be preferred over the other. For instance, females are preferred in the dairy industry, whereas males are favored in the beef industry (Rosenfeld and Roberts, 2004). The sex-allocation hypothesis of Trivers and Willard (1973) predicted that females in the best body condition would tend to produce offspring the gender of which favors the sex of greater variance, namely males. Our current study conducted on different mature size based on breed differences showed that heavier breed such as Kangal produced more males. In conclusion, our results indicate that factors related with insemination do not skew sex ratio in spite of maternal hormonal levels correated with VER and body size at the time of insemination could be a source of variation in sex ratio, however superovulated donors produced higher proportion of female lambs when pregnancy rates of frozen embryo transfer is quite acceptable.

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