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Original article

Influence of sex ratio during multiple pregnancies on productive and reproductive parameters of lambs and ewes

M Avdi [1], MA Driancourt [2*]

[1] *Faculty of Agriculture, Aristotle University of Thessaloniki, GR 540 06 Thessaloniki, Greece;*
[2] *PRMD, Inra, 37380 Nouzilly, France*

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Summary — Because twin (and sometimes triplet) births commonly occur in sheep, interactions between foetuses of opposite sex may occur during life in utero and affect adult productive or reproductive performance. This was investigated by comparing growth, milk production and reproductive features of female lambs born as twins with a sister (FF) or a brother (FM) or born as triplets with two sisters (FFF), one brother and one sister (FFM) or two brothers (FMM). Birth weight, age and weight at puberty and milk production during the first lactation were similar between FF and FM lambs as well as between FFF, FFM and FMM lambs. Most of the reproductive parameters were unaffected by the sex ratio in utero. Ovarian development during the postnatal and prepubertal periods was identical between groups as demonstrated by the lack of differences in follicle-stimulating hormone concentrations and ovarian response to human chorionic gonadotropin. Adult ovulation rate and litter size at first lambing were also not related to sex ratio in utero. In contrast, embryonic mortality measured by the difference between ovulation rate and litter size was significantly ($P < 0.05$) increased in FMM lambs compared to FFF and FFM lambs. The same was observed for FF versus FM lambs. It is concluded that sex ratio in utero may have to be entered in programmes evaluating genetic merit for embryo survival in sheep.

sheep / foetus / sex ratio / ovulation rate / embryonic mortality

Résumé — Influence de la taille et du sex ratio de la portée sur la production et la reproduction des agnelles issues de ces portées. Les naissances doubles et parfois triples sont fré-

* Correspondence and reprints
Tel: (33) 02 47 42 79 21; fax: (33) 02 47 42 77 43; e-mail: driancou@tours.inra.fr

quentes quand la reproduction des ovins est semi-intensive. En cas de gestations multiples, des interactions entre fœtus de sexe opposé peuvent se produire au cours de la gestation et altérer le potentiel adulte de production et/ou de reproduction. L'existence de tels effets a été recherchée en comparant la croissance pondérale, la production laitière et le potentiel de reproduction d'agnelles nées doubles avec une sœur (FF) ou un frère (FM) ou nées triples avec deux sœurs (FFF), un frère et une sœur (FFM) ou deux frères (FMM). Pour un type de naissance donné (double ou triple), les poids de naissance ne sont pas affectés par le sex ratio in utero. C'est également vrai pour l'âge et le poids à la puberté et la production laitière en première lactation. La plupart des paramètres de reproduction n'est pas affectée par le sex ratio in utero. C'est le cas des marqueurs précoces de développement ovarien (concentrations de FSH en période postnatale, ovulation induite par hCG en période prépubère) ainsi que du taux d'ovulation et de la taille de la première portée. En revanche, la mortalité embryonnaire mesurée par la différence entre le taux d'ovulation et la taille de la portée est significativement ($p < 0,05$) augmentée chez les agnelles FMM par rapport aux agnelles FFF et FFM. Une tendance identique est observable quand FF et FM sont comparés. Le sex ratio in utero pourrait être important à introduire dans les programmes de sélection sur la survie embryonnaire.

ovin / fœtus / sex ratio / taux d'ovulation / mortalité embryonnaire

INTRODUCTION

Most breeds of sheep, when conducted in intensive or semi-intensive breeding systems, give birth to twins and sometimes to triplets. Hence, interactions between neighbouring foetuses may occur during pregnancy, particularly when foetuses of opposite sex are side by side in the maternal uterus. Consequences of such interactions can be severe such as observed in freemartins (Lillie, 1916) or more limited such as observed with the intra-uterine position effect (Vom Saal, 1989). Freemartinism, while documented, is very uncommon in sheep (Matejka et al, 1987). In contrast, both in rodents and swine, intra-uterine position effects have been reported. In these species, sex of adjacent siblings in utero affects postnatal morphological (Gandelman et al, 1977; Vom Saal and Bronson, 1978; Vom Saal, 1981) as well as behavioural and reproductive characteristics (Vom Saal and Bronson, 1978, 1980; Rohde Parfet et al, 1990). Whether such effects can be observed in sheep lambing large litters is not documented. The aim of this study was to assess the effects of the composition of the litter on production and reproductive performance of ewe lambs and ewes up to their second lambing.

MATERIALS AND METHODS

Animals and experimental design

Chios ewes ($n = 125$), a breed known for its high natural ovulation rate (Avdi et al, 1988) were hand-mated to Chios rams ($n = 73$) at the beginning of their breeding season (May). At lambing, composition of the litter was recorded and lambs were assigned to specific groups according to litter size and composition of the litter.

Twin lambs could be FF when born with a twin sister of FM when born with a twin brother. Triplets could be FFF when the litter contained three sisters, FFM when it contained two females and one male or FMM when it contained two males and one female.

A number of production parameters were gathered for all the female lambs. They included birth weight, weight at puberty and milk production following weaning of the first litter at 42 days of age. A detailed study of maturation of reproductive function throughout the prepubertal

period was also undertaken. Parameters of the postnatal/prepuberal periods known to be related to adult reproductive performance are follicle-stimulating hormone (FSH) concentrations between 3 and 7 weeks of age (Bindon et al, 1985; Bodin et al, 1988; Sonjaya and Driancourt, 1989) and ovarian response to human chorionic gonadotropin (hCG) at 4–5 months of age (Driancourt et al, 1990). Both parameters were compared in the present study between FF and FM ewe lambs on the one hand as well as between FFF, FFM and FMM ewe lambs on the other hand. At 5 and 7 weeks of age, a single blood sample was obtained by venipuncture from all lambs and stored at –20 °C until assayed for FSH (see later). The ewe lambs were then left undisturbed until 4 months of age when each lamb was given an intramuscular injection of 750 IU of hCG (Chorulon, Intervet, France). Ovulation rate was measured 4 days later by laparoscopy as described by Driancourt et al (1990).

Mature reproductive performance was studied following puberty. Age at puberty was determined following daily inspection of all lambs with vasectomized rams. At the cycle following puberty, all lambs were mated with fertile rams. Ovulation rate was measured 7 days following mating by laparoscopy and all animals were left undisturbed until parturition when litter size was recorded. Embryonic mortality was measured as the difference between ovulation rate at mating and litter size.

Following the first lambing, and weaning of their lambs at 42 days, a large proportion of the experimental ewes (44 FF, 27 FM, 11 FFF, 22 FFM and 12 FMM) was machine-milked twice daily for 145 ± 14 days. Out of a subsample of FF and FM lambs, theses parameters (ovulation rate, litter size and embryonic mortality) were again measured at the second mating when lambs were 2 years old.

FSH assay

Plasma FSH was measured using the homologous NIAMDD assay kit as described by Sonjaya and Driancourt (1989). All samples were measured in a single assay. The intra-assay coefficients of variation were 6.7 and 4.9% for 3.3 and 1.6 ng/mL, respectively.

Statistical analysis

Multiple comparisons (FFF vs FFM vs FMM) were done by ANOVA, while *t*-tests were used to compare features of FF and FM lambs. Percentages were compared by chi-square analysis. All data are expressed as means ± SD.

RESULTS

Unless otherwise specified, the experiment involved 62, 38, 21, 35 and 15 FF, FM, FFF, FFM and FMM females, respectively.

Production parameters

There was no significant effect of the sex of neighbouring siblings on any of the production parameters measured (table I). Whatever the litter size and sex composition of the litter, birth weight, puberty weight and milk production were similar (table I).

Reproductive parameters

None of the parameters measured during the postnatal (FSH concentrations) or prepubertal periods (ovarian response to hCG) differed between FF and FM lambs on the one hand and between FFF, FFM or FMM lambs on the other hand (table II). The proportion of lambs not returning to oestrus following mating was similar in FF and FM lambs (82 and 86%, respectively) as well as between FFF, FFM and FMM lambs (82, 88 and 85%, respectively). The ovulation rate measured 7 days following mating was also similar in all groups (table II). A similar conclusion was valid for prolificacy (table II). Interestingly,

Table I. Production features of female lambs born as twins or triplets with at least one male in the latter (mean ± SD).

Group	*Birth weight (kg)*	*Puberty weight (kg)*	*Milk production following weaning (kg/day)* *
FF ($n = 62$)	3.77 ± 0.5	44.8 ± 6.6	1.44 ± 0.40
FM ($n = 38$)	3.92 ± 0.6	45.2 ± 6.6	1.29 ± 0.28
FFF ($n = 21$)	3.45 ± 0.6	44.5 ± 5.1	1.06 ± 0.29
FFM ($n = 35$)	3.45 ± 0.6	44.6 ± 5.6	1.19 ± 0.25
FMM ($n = 15$)	3.48 ± 0.5	44.4 ± 7.2	1.17 ± 0.40

* Milk production was only measured on 44, 27, 11, 22 and 12 FF, FM, FFF, FFM and FMM ewes, respectively. FF: twin birth with a twin sister; FM: twin birth with a twin brother; FFF: triplet birth with two twin sisters; FFM: triplet birth with one brother and another sister; FMM: triplet birth with two twin brothers.

embryonic mortality measured by the difference between prolificacy and ovulation rate was marginally increased in FM versus FF lambs and significantly ($P < 0.05$) increased in FMM versus FFF or FFM lambs (table II). This was confirmed when a subgroup of FF and FM lambs was studied when 2 years old (table III).

Table II. Reproductive features of female lambs born as twins or triplets with at least one male in the latter (mean ± SD).

Group	*FSH at 5 weeks (ng/mL)*	*FSH at 7 weeks (ng/mL)*	*% of the lambs ovulating after hCG*	*hCG induced ovulation rate*	*Age at puberty (days)*	*Ovulation rate at puberty*	*Prolificacy* *	*Embryonic mortality* *
FF ($n = 62$)	4.4 ± 2.1	4.1 ± 1.5	63	1.5 ± 0.8	262 ± 35	2.1 ± 1.1	1.9 ± 0.7	0.3 ± 0.6
FM ($n = 38$)	4.0 ± 2.0	4.0 ± 1.7	66	1.7 ± 1.1	264 ± 32	2.4 ± 1.2	1.8 ± 0.8	0.6 ± 0.9
FFF ($n = 21$)	2.6 ± 0.7	2.6 ± 1.2	53	1.4 ± 0.7	281 ± 34	2.8 ± 1.1	2.4 ± 1.2	0.5 ± 0.9[a]
FFM ($n = 35$)	3.7 ± 1.7	2.9 ± 1.0	78	1.8 ± 1.3	263 ± 26	2.4 ± 1.1	1.9 ± 0.7	0.5 ± 1.0[a]
FMM ($n = 15$)	3.7 ± 1.9	3.6 ± 0.6	65	1.3 ± 0.7	272 ± 33	2.8 ± 1.0	2.0 ± 0.7	1.0 ± 0.7[b]

[a] versus [b] $P < 0.05$. FF: twin birth with a twin sister; FM: twin birth with a twin brother; FFF: triplet birth with two twin sisters; FFM: triplet birth with one brother and another sister; FMM: triplet birth with two twin brothers. * These parameters were measured on 55, 33, 15, 24 and 12 FF, FM, FFF, FFM and FMM ewes, respectively.

Table III. Ovulation rate, litter size and embryonic mortality of FF and FM lambs (n = 15 each) following the second mating season.

	Ovulation rate	*Litter size*	*Embryonic mortality*
FF	2.57 ± 0.64	2.07 ± 0.61	0.5 ± 0.6[a]
FM	2.86 ± 1.18	1.66 ± 0.49	1.2 ± 1.4[b]

[a] versus [b] $P = 0.05$. Abbreviations as in table I.

DISCUSSION

This is the first report assessing the consequences of the intra-uterine environment in terms of sex ratio on the production potential of sheep. This was achieved by comparing production and reproductive features of FF versus FM females as well as those of FFF versus FFM versus FMM. It should be stressed, however, that in triplets, no information was available on the in utero position of the female lamb(s) relative to the male lamb(s). It was therefore impossible to assign lambs to the OM or 2M condition (ie, surrounded by no males or by two males) as defined earlier by Vom Saal and Bronson (1978, 1980).

The main conclusions of this study are (i) the lack of effects of the sex ratio during intra-uterine life on all production parameters measured and (ii) the lack of effects of the sex ratio during intra-uterine life on most reproductive parameters studied except embryonic mortality which is increased in lambs born twins or triplets with ram lambs.

The first conclusion is in good agreement with earlier reports in rodents (Vom Saal, 1989) and swine (Wise and Christenson, 1992) showing no effect of intra-uterine position on birth weight. Furthermore, in rodents (Vom Saal and Bronson, 1978), as in sheep in the present study, body weight at puberty was also unrelated to intra-uterine environment/position. Influence of intra-uterine environment/position on milk production had not been investigated earlier but the present results demonstrate no relationship between this and milk production, at least during the first lactation.

The second conclusion also largely fits with those reported earlier for rodents and swine. Age of vaginal opening (Vom Saal, 1981) and age at puberty in swine (Rohde Parfet et al, 1990) were unaffected by earlier intra-uterine position, a conclusion in good agreement with the identical ages of puberty of all groups in the present study. Because differences in ovarian function related to intra-uterine position had not been identified in adult rodents (Vom Saal, 1989) or swine (Rohde Parfet et al, 1990) and because earlier markers of ovulatory ability (such as FSH concentrations during the postnatal period or ovarian response to hCG during the prepuberal period) are available in sheep, these markers were used to compare ovarian development during the postnatal and prepuberal periods in FF versus FM lambs as well as between FFF, FFM and FMM lambs. FSH concentrations during the postnatal period are indicative of the status of the developing ovary with high concentrations associated with retarded ovarian development (Sonjaya and Driancourt, 1989). The similarity in FSH concentrations between groups at a specific litter size do not indicate that FM or FMM lambs display retarded ovarian development

compared to the other groups (FF, FFF, FFM). This is further supported later in life by the observations that both the percentage of lambs ovulating following hCG and hCG-induced ovulation rate were identical in all groups. Hence, ovarian development during the postnatal/prepuberal periods is unaffected by the sex ratio in utero.

In rodents (Vom Saal, 1981) and swine (Rohde Parfet et al, 1990), ovulation rate and litter size were unrelated to intra-uterine position, a finding in good agreement with the results of the present study in sheep. The only parameter affected by intra-uterine environment in the present study was embryonic mortality which was increased by the presence of males during intra-uterine life. This was particularly clear when FFF lambs were compared with FMM lambs. FFM lambs also displayed reduced embryonic mortality (although with a larger variation) compared to FMM lambs. This could be related to the unknown location of the male foetus compared to the female one. This effect of intra-uterine position on embryonic mortality had not been reported earlier in rodents because it is impossible in this species to record ovulation rate and litter size in the same animal. However, results in swine (Rohde Parfet et al, 1990) have demonstrated a reduced pregnancy rate (13% less, although this was not significant) and increased embryonic mortality (5% more) for gilts surrounded by two males during pregnancy compared with gilts surrounded by two females. The present study did not try to address the mechanisms involved in these alterations. In rodents, androgens produced by neighbouring foetuses are claimed to be responsible for most of the alterations observed (Vom Saal, 1989). This however does not appear to be the case in swine (Wise and Christenson, 1992). The compounds that may be responsible for the effects observed in the present study remain to be identified. It should be noted that intersexuality was very uncommon in the present sample of ewe lambs.

It can be concluded that there is no need to enter composition of the litter in terms of sex ratio in programmes evaluating genetic merit for milk production or ovulation rate. However, this may be necessary for programmes assessing genetic merit for embryonic mortality.

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