

Uterine morphology and reproductive phenomena in relation to number of embryos at different stages of gestation in prolific sheep

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Abstract

Forty prolific ewes of which half were superovulated with 800 IU PMSG were slaughtered on either d 30, d 70 of gestation or d 3–5 post-partum. At each slaughter date, the ewes were balanced for number of ovulations (1 to >3 at d 30) or embryos (1 to 4 at d 70 and d 3–5 post-partum). Uterine length, width, surface and dry matter (DM); embryonic weight and DM; plasma progesterone at d 15 and 30 of gestation; and protein concentration in allantoic fluids were measured. Superovulated ewes averaged 5.8 CL vs. 3.1 CL for the control. On d 15 of gestation, plasma progesterone concentration increased from 4.87 ng/ml in ewes with 2 CL to 8.02 ng/ml for those with >7 CL. The corresponding figures at d 30 were 4.19 and 9.44 ng/ml, respectively. Embryonic losses increased from 16.5% in ewes with 2 CL to 67.9% in those with >7 CL. Length of uterine horns increased by 9.2, 7.7 and 4.1 cm for each increase in number of embryos present at the three slaughter dates, respectively. On d 30, only embryo DM showed a significant ($P < 0.05$) increase with increased number of embryos present. On d 70, length, width and uterine surface increased linearly with increase in number of embryos, however, little difference was observed between ewes carrying three and four embryos. Ewes slaughtered after they had lambed one, two or three lambs had significantly smaller and narrower uteri than those with four lambs. It is concluded that, in prolific as in non-prolific sheep the space available seems to be adequate for development of all embryos surviving the critical first 30 days of pregnancy.

Key words: Uterus; Superovulation; Embryo; Mortality; Prolific sheep

1. Introduction

The number of lambs born is a function of ovulation rate and embryonic survival which is determined to a great extent by the physical and physiological capacity of the uterus. In non-prolific breeds of sheep, where the number of lambs born is often one or two and rarely three, the uterine capacity is seldom a determining factor for the number or size of young born. This situation

may be different in prolific breeds with high ovulation rate, for which ovulation rate (even after early embryonic loss) could be higher than the ability of the uterus to accommodate the surviving embryos. The limiting effect of the uterine space may have a greater impact when prolificacy is introduced abruptly in originally non-prolific breeds such as in the case of introducing a major gene for prolificacy, for example, the Booroola gene. Despite the apparent importance of the subject (Leymaster and Bennett, 1990), few reports were pub-

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lished on the limiting effect of the uterus since the classical work of Wallace (1948).

Lawson (1971) transferred five fertilised ova each to mature Romney March, Suffolk and Finnish Landrace ewes, breeds with natural ovulation rates of 1.5, 2.1 and 2.5, respectively. The litter size observed at lambing was 2.8, 2.9 and 3.3 lambs, respectively. The authors suggested that the lower prolificacy of Romney March ewes under natural conditions is a result of low ovulation rate, and that their uterine capacity compares favourably with ewes of more prolific breeds. Working with twin-ovulating ewes from Romney and Romney crosses, Meyer (1985) showed that the crosses produced between 4 and 34% more lambs than the pure Romneys, indicating that their uterine environment and/or capacity was probably superior to that of pure Romneys.

The purpose of the present study was to measure the physical characteristics of the uterus of prolific sheep carrying different loads of embryos to determine to what extent the uterus limits litter size at different stages of gestation. The data also provided information on some reproductive characteristics of prolific sheep during gestation.

2. Materials and methods

The animals used in this study were 19 Finnsheep and 21 Arcott Rideau (a synthetic breed with approx. 40% Finnsheep ancestry) mature ewes, varying in age between 2 (21 ewes) and 5 years (8 ewes). These ewes were born at the Animal Research Centre in Ottawa and were transferred to the La Pocatière Experimental Farm in December 1989. All of them had one lambing at La Pocatière in 1990 before being involved in this experiment.

In a preliminary experiment, 30 prolific ewes were synchronized using vaginal sponges impregnated with 60 mg medroxyprogesterone acetate (Veramix, Upjohn). At sponge withdrawal, 14 d after insertion, ewes were superovulated with 800 IU of PMSG (Equinex, Ayerst) to evaluate the response of these prolific ewes to that specific dose. Number of corpora lutea

(CL) present was recorded by laparoscopy 9 d following sponge withdrawal. These ewes were allowed one estrous cycle to recuperate before being included in the main study with ten other ewes that had not been synchronized before.

Half the ewes in both these groups ($n=20$) were then superovulated with the previously described treatment, whereas the other ewes served as controls ($n=20$). The objective of superovulation was to insure sufficient numbers of embryos to challenge the uterus. All the ewes were mated to Arcott Canadian rams and the day of mating was recorded as d 0 of gestation. Seven days after mating, laparoscopy was performed and the ewes were allocated according to the number of CL observed, in such a manner that ewes with 2, 3, 4, 5, and more than 5 CL would be present in approximately equal numbers for each of the following slaughter ages, d 30 or 70 of gestation or d 3–5 post-partum.

Blood samples were taken from all ewes on d 15 and d 30 of gestation to determine serum progesterone level by radioimmunoassay (RIA). The RIA procedures applied in the present study, as well as the extract efficiency, assay sensitivity, and intra- and inter-assay coefficient of variation are as reported in detail by Guilbault et al. (1988). The reason for determining the progesterone level was to relate it to other traits studied such as embryo survival and uterine measurements. To establish the number of developing embryos, the ewes to be slaughtered on d 70 of gestation and d 4 post-partum were scanned (echography) on d 60, using Aloka model SSD-210DX ultrasonic scanner device (Tokyo, Japan). According to the results of that test the ewes were reallocated equally among the two remaining times of slaughter.

At the slaughter house, the carcasses were weighed hot and the reproductive tract was removed, stored on ice and transferred to the Lennoxville Research Station for analysis. The entire tract was weighed, the ovaries removed and the number of CL on each ovary counted. The uterine horns were dissected opposite to the mesometrium to expose the embryos. To obtain an approximate estimation of the uterine proteins secreted by ewes slaughtered on d 30 and 70 of gestation, a sample of the allantoic fluids was taken using a sterile syringe. Protein determinations on the fluids were done according to Pollard et al. (1978) using a Bradford Protein Assay Kit (Bio-Rad Lab., Mississauga, Ont.). The number of embryos present in each uterine horn was

recorded. On d 30, the combined weight of all the embryos was taken, while on d 70 the weight of individual embryos was recorded then pooled. Weight of the litter was calculated from individual embryonic weights and weight of the placentae. For the ewes which lambled, the individual weight of each lamb was taken. The empty uterus of each ewe was weighed, then the uteri were placed on strong waterproof paper and length and width (at 1/3 and 2/3 of length) of the uterine horns and length of uterine body were measured. The surface of each uterus was measured from a tracing using an electric planimeter (Planix, Tamaya, Tokyo, Japan). Uteri from the three slaughter periods and embryos from the first two periods were homogenized and their dry matter content determined by freeze drying the entire sample.

2.1. Traits studied and statistical analysis

Data were analyzed using SAS, GLM procedure (SAS, 1985). Different models were used according to the traits analyzed. Because some variables showed abnormal distributions, the most appropriate transformation was applied to convert the data to a normal distribution. Effect of superovulation on the number of CL present on the ovaries and embryonic loss (number of CL – number of surviving embryos) was analyzed in a 3 × 3 factorial with previous and present superovulations and age of ewe (2, 3 or > 3 yr old) and their interaction as the main factors. The covariate of litter size at the last lambing was included in the model but since its effect on the results was negligible, it was excluded from the final analysis.

Progesterone concentration and ovulation loss were studied as a function of number of CL observed at laparoscopy. Numbers of CL were classified into 2, 3, 4, 5–6, 7–8 and > 8. The accuracy of laparoscopy in determining ovulation rate, and of echography in determining litter size was measured by Pearson's correlation coefficient. The effect of number of embryos present on uterine physical characteristics and allantoic fluid proteins was studied within slaughter periods. Weight of the carcass was included as a covariate in the model. In ewes slaughtered after lambing, the number of days from lambing to slaughter was included as a covariate. Linear and parabolic regression analyses were conducted to establish the relationship between number of embryos (or lambs) and the measurements studied (SAS, 1985).

3. Results and discussion

3.1. Effect of superovulation

Average number of CL in the 23 ewes which were superovulated in the preliminary trial was 5.0. The range extended from 2 to 12 CL with 16 ewes (70%) ovulating between 3–5 CL and one each ovulating 8, 10 and 12. In the main trial, ewes that were superovulated averaged 5.8 CL (range 3–12), compared to 3.1 CL (range 2–6) for the control ($P < 0.01$). Ewes which were superovulated twice (in the primary and main trial) had 2.1 less CL ($P < 0.05$) than superovulated ewes, which were not superovulated in the primary. Likewise, the ewes that were not superovulated in the two cycles had a slight tendency to ovulate more than those superovulated in the first cycle (Table 1). The interaction on number of CL between the superovulation for first and second times, although nonsignificant, indicated that the first superovulation affected ovulation adversely following the second superovulation. A similar adverse effect of repeated superovulation was reported by Driancourt (1987) at a 3-week interval and Hanrahan and Quirke (1982) at yearly intervals.

Although there is much literature on the effect of PMSG given in doses ranging from 500 to 2500 IU in inducing superovulation in non-prolific sheep (reviewed by Smith, 1988), few reports are available on the proper level for superovulating prolific sheep. Rainio (1991) superovulated Finnsheep with 1200, 1500 and 1800 IU doses of PMSG, with the corresponding ovulation means being 7.5, 10.9 and 12.3 CL, respectively. Quirke et al. (1987) used 375, 750 and 1500 IU of PMSG on Finnsheep and reported 3.53, 5.01 and 10.36 CL, respectively. In the present study, because superovulation was used only as a means for challenging the uterus, an 800 IU dose was judged sufficient, and the preliminary trial superovulation was conducted to confirm that.

3.2. Accuracy of laparoscopy and echography

Since observations were made on ovulation rate by examining the number of CL using a laparoscope and also by dissecting the ovaries after slaughter, it was possible to evaluate the accuracy of the technique of laparoscopy with sheep varying widely in ovulation rate. Pearson's coefficient of correlation was 0.95 indicating a high ($P < 0.01$) accuracy of the laparoscopy. The relationship between number of embryos recorded

Table 1

Least-squares means and SEM of corpora lutea in the second estrous cycle of control and superovulated ewes according to whether they had been previously superovulated

Main superovulation	Preliminary superovulation				Average ± SEM
	superovulated		control		
	No.	mean	No.	mean	
Superovulated	12	4.8 ^a	8	6.9 ^c	5.8 ± 0.4
Control	11	2.9 ^b	9	3.3 ^{ab}	3.1 ± 0.4
Average ± SEM		3.8 ± 0.4		5.1 ± 0.4	

^{abc}Means followed by different letter are statistically different ($P < 0.05$).

by ultrasonic real time scan taken on d 60 of pregnancy and the actual number of embryos recorded after slaughter and at lambing was also examined. The correlation coefficient was 0.84, ($P < 0.01$). The accuracy of laparoscopy and echography in predicting the number of ovulations and embryos have been reported in numerous studies (White et al., 1984). It seems that when the number is rather small the accuracy is higher, however examining the relationship between observed and actual data indicates that the discrepancy occurred at all levels of ovulations in the present study.

3.3. Effect of number of ovulations

The effect of number of ovulations on the level of progesterone on d 15 and d 30 after ovulation, protein content of the allantoic fluid and ovum and embryonic loss is presented in Table 2. Ewes with more than seven ovulations had significantly higher serum progesterone concentration ($P < 0.05$) than those with two, three or four ovulations (also 5–7 ova on d 30).

As expected, progesterone concentrations increased with increased number of ovulations ($b = 0.67$, $P < 0.03$, and $b = 0.83$, $P < 0.02$, at d 30 and d 70, respectively). Eastwood et al. (1976) determined progesterone concentrations at 20, 40 and 60 d of gestation in Romney and Border Leicester × Romney ewes superovulated with PMSG. The number of ovulations increased from 1 to 6 and the corresponding progesterone concentrations were 3.3 and 9.3 ng/ml. There was an increase of about 1 ng/ml for every increase in the number of CL present. There was also a significant ($P < 0.001$) increase in progesterone concentration with stage of gestation (from 20 to 60 d), contrary to

what was observed in the present study (from 15 to 30 d), probably as a result of differences in the stage of gestation covered in the two studies. Working with Finnsheep × Dorset horn ewes superovulated with PMSG, Rhind et al. (1980) reported higher mean progesterone concentrations at 40 and 70 d of gestation (ranging from 6.6 ng/ml for ewes with 3 CL at 40 d to 15.8 ng/ml for ewes with > 5 CL at 70 d).

Concentration of allantoic proteins, secreted by the uterus for various pregnancy-related functions (Newton et al., 1989), were similar in ewes with 3, 4 and 5–7 ovulations, and higher than those with two or seven ovulations. These differences were, however, nonsignificant. Level of allantoic proteins decreased drastically, however, from d 30 to d 70 of gestation (mean 0.73 vs. 0.10 mg/ml, respectively, Tables 3 and 4). Concentration and not volume was determined, and although the amount available for each embryo did not seem to change, the total volume secreted by the ewe increased proportionate to the number of embryos present, agreeing with findings of Arthur (1969) and Newton et al. (1989).

Ova loss (result of fertilization failure and/or embryonic mortality) was approx. 17% for ewes with two and three ovulations, approx. 37% for ewes with up to seven ovulations, and 68% for ewes with ovulations greater than seven. Relationship between number of ovulations and number of embryos (or lambs for ewes slaughtered after lambing) is presented in Fig. 1 for superovulated and control ewes. Ten ewes had five or more ovulations, but only two carried quintuplets and four had quadruplets, which may suggest that biological limits of these ewes were near that range. Sta-

Table 2

Effect of number of ovulations on serum progesterone, allantoic proteins and embryonic loss

Traits studied	Number of ovulations					SEM [‡]
	2	3	4	5–7	> 7	
No. of animals	3	17	10	5	5	
Progesterone on d 15 (ng/ml) [†]	4.87 ^b	4.64 ^b	5.14 ^b	8.18 ^a	8.02 ^a	0.98–0.41
Progesterone on d 30 (ng/ml) ^{††}	4.19 ^b	4.74 ^b	4.46 ^b	6.01 ^b	9.44 ^a	0.87–0.36
Allantoic proteins (mg/ml)	0.09	0.45	0.36	0.60	0.09	0.39–0.15
No. of ovulation lost [†] (percent of ovulations)	0.3 ^d (16.5)	0.5 ^{cd} (17.7)	1.4 ^{bc} (36.0)	2.4 ^b (38.7)	7.2 ^a (67.9)	0.53–0.22

^{a–d}Means followed by different superscripts are significantly different. ($P < 0.05$).

[†]Significant linear regression on number of ovulations ($b = 0.67 \pm 0.18$, $P < 0.03$).

^{††}Significant linear regression on number of ovulations ($b = 0.83 \pm 0.20$, $P < 0.02$).

[‡]SEM associated with the observation with the least and most numbers, respectively.

tistically, the relation between number of ovulations and the ensuing surviving embryos was linear for ovulations up to seven, fitting a curve to the entire data indicated that 9.4 ovulations was the maximum for obtaining highest number of embryos (or lambs).

According to Rhind et al. (1980) ewes with three, four or five ovulations lost between 24 and 30% of their embryos, compared to 57 to 72% for those with six to nine ovulations. Land and Wilmut (1977) studied embryo survival in Blackface and Finnsheep ewes, which had received either seven or 14 embryos, and found embryo survival rates of 28 and 4% for Blackface ewes, and 10% for Finnsheep receiving 14 embryos. They concluded that the difference in survival rate was directly related to ovulation rate of the recipient ewe. These results are in general agreement with those presented here.

3.4. Embryonic mortality at different stages of gestation

Since the ewes were distributed among the three slaughter periods according to number of ovulations, it was possible to calculate ovulation loss at the different stages. Table 3 shows that most of the loss occurred before d 30 of gestation. It seems that the first 30 days are the most critical in superovulated and prolific sheep, similar to what was observed in swine (Marlowe and Smith, 1971; Dufour and Fahmy, 1975). This agrees with Edey (1969) reviewing numerous studies, and

more recently Wilkins et al. (1985) working on prolific and non-prolific sheep. Kelly et al. (1989) reported that little or no embryonic mortality occurs after 30 d in adequately fed Merino sheep bearing twins. Wilkins et al. (1985) agreed with the findings of Kelly for non-prolific sheep, but reported embryonic mortality in excess of 10% in prolific Booroola Merino. Similar findings can be drawn from the present data when the observation for one ewe which had ovulated ten ova, but lost them all, was removed from the analysis.

3.5. Embryo migration and position

Examining the number of CL on each ovary and the number of embryos in the corresponding horns of the ewes sacrificed on d 70 of gestation, provided information on the migration of embryos between the two uterine horns. Migration could be confirmed in at least six cases of the 14 available. In five of these six cases one ovary did not ovulate at all, yet one embryo and in one case two embryos migrated to the uterine horn opposite to the ovary. In superovulated ewes with more than 5 CL, embryos were lost more from the side with the largest number of ovulations compared to the other side. Wallace (1948) reported that in all the cases where multiple ovulation occurred on one ovary, one embryo migrated to the other horn. Scanlon (1972) studied transuterine migration on a large number of ewes with one or two ovulations. He showed that in ewes with one ovulation, 8% of the fetuses were present

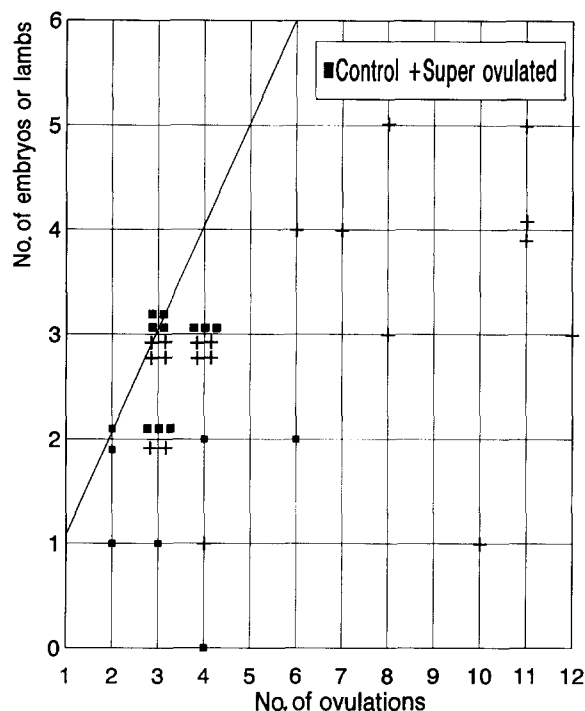


Fig. 1. Relationship between number of ovulations and number of embryos or lambs born for control and superovulated ewes. The line in the body of the figure represents litters with no loss.

Table 3
Ovulation loss and embryonic mortality for the three slaughter periods

	Slaughter period		
	gestation		postpartum
	day 30	day 70	day 4
Number of ovulations	59	60	62
Number of surviving embryos	34	37	35
Ova loss and embryonic mortality (%)	43.3 [†]	38.3	43.6

[†]When a ewe which had 10 ovulations and no embryos was removed, this figure would be 30.6%.

in the opposite uterine horn, while in 88% of the ewes with two ovulations on the same ovary, evidence of transuterine migration was observed.

Of the 36 embryos observed on d 70 of gestation, 23

(64%) were presented in the normal lambing position, i.e., the head facing the cervix, while in 13 (36%) the head was facing in the direction of the ovary. Since previous observations with prolific sheep at this station indicated that the number of lambs in abnormal position at lambing was rather small, it seems that the fetuses can change position, sometimes between d 70 of gestation and parturition.

3.6. Effect of number of embryos on the uterine physical measurements

Tables 4–6 present the means for traits studied on d 30, and d 70 of gestation and d 3–5 post-partum, respectively. There was no ewe carrying one embryo and only one carrying four embryos on d 30 of gestation, therefore, the comparisons are mainly between ewes carrying two and three embryos. Ewes carrying three embryos had 14% longer but 5–10% narrower uteri than ewes carrying two embryos ($P < 0.05$). Surface of the uterus was similar in the two cases (262 vs. 257 cm²). Only for DM content of the embryos and uterine horns did the difference reach significance, with ewes carrying three embryos showing higher values.

On d 70 of pregnancy, ewes carrying three or four embryos showed similar uterine measurements. Ewes with three embryos had 10.5% longer and 16% wider uteri than those carrying two embryos which in turn had 6% longer and over 50% wider horns than ewes carrying only one embryo. The surface of the uterus increased linearly with the number of embryos carried (Table 5).

On d 4 post-partum (one ewe on d 3 and two on d 5) empty uteri had already shown a marked regression reaching the size they had attained on d 70 of gestation when they were still carrying embryos (Table 6). Ewes giving birth to one, two or three lambs had similar uterine dimensions, which were generally smaller than those giving birth to four lambs, but the differences were again non-significant. Present results indicated that uterine involution in sheep is a fairly rapid process, and measurements taken as early as 3 d after lambing are probably not indicative of the real physical state of the uterus at the end of pregnancy.

Wallace (1948) reviewed the early research on uterine characteristics conducted before 1948 on non-prolific sheep. His own detailed work on ewes carrying single and twin embryos indicated that uterine walls were between 25 and 44% heavier in twin- than in

Table 4
Least-squares means and SEM for the traits on d 30 of pregnancy

Traits	Number of embryos				
	2		3		4
	mean	SEM	mean	SEM	mean
Number of ewes	4		7		1
Uterine measurements					
Length of horns [†] (cm)	52.4	4.6	60.0	3.6	72.1
Length of body (cm)	3.5	0.4	4.2	0.3	4.4
Width upper 1/3 of horns (cm)	4.3	0.3	3.9	0.2	3.1
Width lower 1/3 of horns (cm)	5.0	0.4	4.8	0.3	3.0
Surface (cm ²)	257 ^a	12	262 ^a	10	168 ^b
Dry matter (%)	14.9 ^a	0.2	15.7 ^b	0.3	15.6 ^{ab}
Total weight of litter [†] (g)	32.0	3.1	35.6	3.4	26.4
Avg. weight of embryos [†] (g)	16.6 ^a	2.4	12.2 ^{ab}	1.0	6.6 ^b
Dry matter of embryos [†] (%)	3.5 ^a	0.2	4.2 ^b	0.2	6.2 ^c
Proteins in allantoic fluids (mg/ml)	0.71	0.29	0.68	0.23	1.21

^{a,b}Means followed by different superscripts are significantly different ($P < 0.05$).

[†]Include placenta.

[‡]Linear regression on number of embryos, $b = 9.23 \pm 4.06$ ($P < 0.05$).

Table 5
Least-squares means and SEM for the traits at d 70 of gestation

Traits	Number of embryos				SEM [†]	b [‡] ± SE
	1	2	3	4		
Number of ewes	2	5	4	3		
Uterine measurements						
Length of horns (cm)	68.4	75.9	81.7	80.2	5.9–4.1	7.7 ± 2.3**
Length of body (cm)	8.5	6.5	9.0	7.7	1.1–0.8	
Width upper 1/3 (cm)	6.8	12.9	15.1	15.2	2.9–2.0	2.3 ± 1.0*
Width lower 1/3 (cm)	13.0	16.1	18.7	18.8	1.8–1.4	2.0 ± 0.6*
Surface (cm ²)	796 ^a	895 ^a	1205 ^{ab}	1410 ^b	142–98	225 ± 49**
Dry matter (%)	16.9 ^a	11.8 ^b	10.6 ^b	11.5 ^b	0.6–0.4	–1.4 ± 0.5*
Total weight of litter (g)	166	408	544	756	79–54	
Avg wt. of embryos (g)	159	190	192	173	21–15	
Dry matter of embryos (%)	9.2	8.6	9.4	9.0	0.6–0.3	
Protein in allantoic liquid (mg/ml)	0.07	0.12	0.11	0.07	0.04–0.02	

^{a,b}Means followed by different letters are significantly different ($P < 0.05$).

[†]SEM for the observations with least and most numbers, respectively.

[‡]Significant linear regression on number of embryos.

* $P < 0.05$; ** $P < 0.01$.

single-bearing ewes between 28 and 140 d of gestation. Little has been published since on the physical characteristics of the uterus in sheep and particularly prolific breeds. Fahmy (1992) reported on weights and meas-

urements of uteri in non-pregnant prolific and non-prolific ewes between the ages of 6 and 11 months of age. Although the difference in ovulation rate between the genetic groups was significant, the differences in

Table 6
Least-squares means and SEM for the traits at 3–5 days postpartum

Traits	No. of lambs born				SEM	$b^{\dagger} \pm SE$
	1	2	3	4		
Number of ewes	2	3	7	2		
Uterine measurements						
Length of horns (cm)	75.3	76.1	83.6	84.8	9.4–5.2	
Length of body (cm)	4.9	5.8	5.5	6.9	0.8–0.4	
Width upper 1/3 (cm)	11.8	11.4	13.4	18.0	2.1–1.2	1.8–0.9*
Width lower 1/3 (cm)	15.8	14.3	16.8	23.3	3.1–1.7	3.60 ± 1.17*
Surface (cm ²)	689	708	876	1147	178–97	142 ± 67 [†]
Dry matter (%)	14.9	15.6	15.0	14.8	0.7–0.4	
Total wt. of litter (kg)	3.2 ^a	6.8 ^b	9.2 ^c	13.9 ^d	1.0–0.6	
Avg weight of lambs (kg)	3.2	3.3	3.2	3.0	0.4–0.2	

^{a–d}Means followed by different letters are significantly different ($P < 0.05$).

[†] $P = 0.057$; * $P < 0.05$.

[‡]Linear regression on number of embryos.

uterine measurements were small and non-significant.

Weight of individual embryos (or lambs) was similar in litters of different sizes at the three stages studied. This was also shown by Wallace (1948) reporting on ewes carrying single and twin embryos and slaughtered at 26, 56, and 112 days of gestation.

4. Conclusions

There is a biological limit beyond which the uterine space can not expand. This limit can differ in different breeds, and at different stages of the reproductive life. Knowledge of the time when the uterine space starts to control the number of embryos is important for superovulation and embryo transfer studies and applications. When the number of embryos and the space they require to develop are within the biological limit, less of a chance exists for these embryos to be lost, unlike when the physical capacity is reached and the embryos have to compete for the existing limited space. The expected relationship therefore is curvilinear, with the uterus expanding proportionally to the increase in number of embryos until it reaches its biological capacity and stays constant thereafter, with the embryos surviving at that stage competing for the available space and nutrients. Due to the limited number of animals available, a significant quadratic relationship could not be

fitted to these data except for DM in uterine horns on d 70 of gestation.

In the pig, most of the embryonic loss occurs before 30 days of gestation, long before the available uterine space can be a determining factor. It seems from the present study with limited data, and others reported previously with larger data, that up to d 30 of gestation is also a critical time in sheep. Most of the embryos destined to be lost would be lost by then. It is difficult to determine whether loss is due to embryonic mortality or failure to fertilize, especially in superovulated ewes with high concentrations of progesterone. However, embryonic mortality is a more likely explanation since fertilization rate in farm animals is fairly high (Hancock, 1962). At that stage of pregnancy the uterine carrying capacity does not seem to be a cause for embryonic mortality. However, the present data on prolific sheep confirm observations made earlier that if more than 10% of embryo loss occurs after d 30 of gestation, uterine capacity may then be a limiting factor.

It is concluded that the space available seems to be adequate for development of most of the embryos surviving the critical first d 30 of pregnancy since embryonic weight and average weight of the lambs did not vary between groups of different ovulation sizes. Because most of the embryonic mortality in prolific sheep had already occurred by d 30 of gestation, it is probable that in sheep as in pigs, the limiting capacity

is not likely a physical space but rather is an embryo-maternal relationship which develops in the first days of gestation.

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References

- Arthur, G.H., 1969. The fetal fluids of domestic animals. *J. Reprod. Fertil.*, 9: 45–52 (Suppl.)
- Driancourt, M.A., 1987. Ovarian features contributing to the variability of PMSG-induced ovulation rate in sheep. *J. Reprod. Fertil.*, 80: 207–212.
- Dufour, J.J. and Fahmy, M.H., 1975. Embryonic mortality and development during early pregnancy in three breeds of swine with purebred and crossbred litters. *Can. J. Anim. Sci.*, 55: 9–15.
- Eastwood, K.C., Payne, E., Fairclough, R.J. and McDonald, M.F., 1976. Plasma progesterone concentration during pregnancy in Romney and Border Leicester × Romney ewes after gonadotropin treatment. *NZ J. Agric. Res.*, 19: 271–276.
- Edey, T.N., 1969. Prenatal mortality in sheep: a review. *Anim. Breed. Abstr.*, 37: 173–190.
- Fahmy, M.H., 1992. Effect of diet on ovarian and uterine measurements of ewe lambs with or without Finnsheep breeding. *Small Rumin. Res.*, 7: 271–276.
- Guilbault, L.A., Roy, G.L., Grasso, F. and Matton, P., 1988. Influence of pregnancy on the onset of oestrus and luteal function after prostaglandin-induced luteolysis in cattle. *J. Reprod. Fertil.*, 84: 461–468.
- Hancock, J.L., 1962. Fertilization in farm animals. *Anim. Breed. Abstr.*, 30: 285–310.
- Hanrahan, J.P. and Quirke, J.F., 1982. Selection on ovulation rate in sheep aided by the use of superovulation and egg transfer. *Proc. 1st World Cong. Sheep and Cattle Breed.*, H.A. Barton and W.C. Smith (Eds.), 2: 329–335.
- Kelly, R.W., Wilkins, J.F. and Newnham, J.P., 1989. Fetal mortality from day 30 of pregnancy in Merino ewes offered different levels of nutrition. *Aust. J. Exp. Agric.*, 29: 339–342.
- Land, R.B. and Wilmut, I., 1977. The survival of embryos transferred in large groups to sheep of breeds with different ovulation rates. *Anim. Prod.*, 24: 183–187.
- Lawson, R.A.S., 1971. Potential uterine capacity in low, medium and highly fertile breeds of sheep. *Vet. Rec.*, 88: 210.
- Leymaster, K.A. and Bennett, G.L., 1990. Models of litter size and their consequences. *Proc. 4th World Cong. Genetics Appl. Livest. Prod.* XVI: 299–305. Edinburgh, Scotland.
- Marlowe, T.J. and Smith, J.I., 1971. Early prenatal death loss in pigs. *J. Anim. Sci.*, 33: 203 (Abstr.)
- Meyer, H.H., 1985. Breed differences in ovulation rate and uterine efficiency and their contribution to fecundity. In: R.B. Land and D.W. Robinson (Eds.), *Genetics of Reproduction in Sheep*. Butterworth, London, pp. 185–192.
- Newton, G.R., Hansen, P.J., Bazer, F.W., Leslie, M.V., Stephenson, D.C. and Low, B.G., 1989. Presence of the major progesterone-induced proteins of the sheep endometrium in fetal fluids. *Biol. Reprod.*, 40: 417–424.
- Pollard, H.B., Menard, R., Brandt, H.A., Pazoles, C.J., Creutz, C.E. and Ramu, A., 1978. Application of Bradford's protein assay to adrenal gland sub cellular fractions. *Anal. Biochem.*, 86: 761–763.
- Quirke, J.F., Meyer, H.H., Lahlou-Kassi, A., Hanrahan, J.P., Bradford, G.E. and Stabenfeldt, G.H., 1987. Natural and induced ovulation rate in prolific and non-prolific breeds of sheep in Ireland, Morocco and New Zealand. *J. Repr. Fertil.*, 81: 309–316.
- Rainio, V., 1991. PMSG-Dose in Finnsheep embryo production. *Theriogenology*, 35: 261.
- Rhind, S.M., Robinson, J.J., Fraser, C. and McHattie, I., 1980. Ovulation and embryo survival rates and plasma progesterone concentrations of prolific ewes treated with PMSG. *J. Reprod. Fertil.*, 58: 139–144.
- SAS, 1985. *User's Guide. Statistics. (5th Edn.)* SAS Inst. Inc. Cary, NC, USA.
- Scanlon, P.F., 1972. Frequency of transuterine migration of embryos in ewes and cows. *J. Anim. Sci.*, 34: 791–794.
- Smith, C.L., 1988. Superovulation in sheep. *Compendium on Continuing Education for the Practicing Veterinarian*, 10: 1415–1423.
- Wallace, L.R., 1948. The growth of lamb before and after birth in relation to the level of nutrition. Part II. *J. Agric. Sci. (Camb.)*, 38: 243–302.
- White, I.R., Russel, A.J.F. and Fowler, D.G., 1984. Real-time ultrasonic scanning in the diagnosis of pregnancy and the determination of fetal numbers in sheep. *Vet. Rec.*, 115: 140–143.
- Wilkins J.F., Fowler, D.G., Bindon, B.M., Piper, L.R., Hall, D.G. and Fogarty, N.M., 1985. Measuring foetal loss with real time scanning. *Anim. Prod. Aust.*, 15: 768.
- Fahmy, M.H., Castonguay, F. et Laforest, J.-P., 1994. La morphologie de l'utérus et la reproduction en fonction du nombre d'embryons présents à divers stades de gestation chez la brebis prolifique. *Small Rumin. Res.*, 13: 159–168.

Résumé

Fahmy, M.H., Castonguay, F. et Laforest, J.-P., 1994. La morphologie de l'utérus et la reproduction en fonction du nombre d'embryons présents à divers stades de gestation chez la brebis prolifique. *Small Rumin. Res.*, 13: 159–168.

Quarante brebis prolifiques dont la moitié avaient été suroovulées avec 800 UI de PMSG ont été abattues soit au j 30 ou au j 70 de gestation,

ou 3 à 5 j après la parturition. Une laparoscopie après l'accouplement et une échographie au j 60 de gestation ont permis de répartir les brebis en différents groupes pour chacune des trois périodes d'abattage en fonction du nombre d'ovulations (1 à > 3 au j 30) ou d'embryons observés (1 à 4 au J 70 et j 3–5 après la parturition). La longueur, la largeur, la surface et le contenu en matière sèche (MS) de l'utérus ont été notées. La concentration de progesterone au j 15 et 30 de gestation et la concentration en protéines du liquide allantoïque étaient aussi déterminés. Les brebis ayant subi le traitement surovlant avaient en moyenne 5,8 corps jaunes (CL) comparativement à 3,1 CL pour les témoins. Au j 15, la concentration plasmatique de progestérone passait de 4,87 ng/ml pour les brebis présentant 2 CL à 8,02 ng/ml pour celles ayant > 7 CL. Au j 30 les chiffres correspondants étaient 4,19 et 9,44 ng/ml, respectivement. La mortalité embryonnaire augmentait de 16,5, à 67,9% pour les brebis présentant 2, et > 7 CL, respectivement. La longueur de la corne utérine augmentait de 9,2, 7,7 et 4,1 cm pour chaque embryon additionnel aux trois jours d'abattages, respectivement. Le contenu en MS des embryons était la seule variable mesurée qui augmentait avec l'accroissement du nombre d'embryons présents, au j 30 de gestation. Au j 70, une augmentation linéaire de la longueur, de la largeur et de la surface de l'utérus en fonction du nombre d'embryons a été observée. Cependant, les différences entre brebis ayant 3 et 4 embryons étaient relativement faibles. Les brebis abattues après avoir mis bas à 1, 2 ou 3 agneaux avaient des utérus significativement plus courts et plus étroits que celles ayant mis bas à 4 agneaux. Nous pouvons conclure que, chez les races de moutons prolifiques comme chez les nonprolifiques, l'espace utérin nécessaire semble adéquat pour le développement de tous les embryons qui survivent après les 30 premiers jours critiques de la gestation.