OVARIAN FOLLICULAR DYNAMICS IN LINES OF SHEEP (FINN, MERINOS) SELECTED ON OVULATION RATE

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ABSTRACT

Ewes from selected lines of sheep from each of two breeds, Finns (high ovulation rate, low ovulation rate and control lines with respective ovulation rates of 5.4, 2.7 and 3.3) and Merinos (T Merinos selected for increased ovulation rate and control Merinos with respective ovulation rates of 1.9 and 1.2) were used to examine how selection to alter ovulation rate had altered follicle development. Ovarian antral follicles were counted, measured, classified as nonatretic or atretic (more than five pyknotic bodies). The growth of ovulatory follicles in vivo, followed by repeated follicle ink marking, also was compared in the three lines of Finns. Regardless of breed, ewes selected for high ovulation rate had a similar number of antral follicles and a similar extent of atresia compared with their controls. Alterations induced by selection were located in the last stages of folliculogenesis. T Merinos exhibited a lower proportion of atretic follicles among follicles >3 mm and a larger diameter of the largest healthy follicle when preovulatory follicles were excluded. High-line Finn ewes recruited more follicles, which produced smaller preovulatory follicles, each containing a smaller number of granulosa cells compared with either the low- or control-line ewes. Hence, physiological selection for high ovulation rate raised it by different methods in Merino than in Finn ewes.
(Key Words: Sheep, Follicles, Ovulation Rate, Selection, Granulosa Cells.)

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Introduction

Heritability of ovulation rate is limited in nonprolific breeds of sheep (.15, Piper et al., 1980) but it is markedly higher in prolific breeds, particularly the Finn (.5, Hanrahan and Quirke, 1985). Hence, successful selection for high ovulation rate has been undertaken in both breeds, resulting in a divergence of about .8 ovulations after 30 yr of selection between prolific T Merinos and control Merinos (Newton-Turner, 1978) and a superiority of about two ovulations in the prolific high-line Finn compared with control-line Finn (Hanrahan and Quirke, 1985); with the line selected against ovulation rate, a slight decrease in ovulation rate (about .5 ovulations less in low Finn compared with controls) has been recorded (Hanrahan, 1987).

The aim of this study was to use ewes of Merino and Finn selection lines to assess whether 1) the overall number of growing follicles was affected by selection or 2) the alterations induced by selection were limited to the population of large follicles. The first hypothesis is supported by some breed comparisons in which prolific ewes had more follicles than their controls (Romanov: Cahill et al., 1979; D'Man: Lahlou-Kassi and Mari-

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ana, 1984). In the latter case, ewes carrying the Booroola prolificacy gene were compared with noncarriers of the same strain (Driancourt et al., 1985; McNatty et al., 1986).

Materials and Methods

Animals. Eleven high-, 10 low- and 7 control-line mature (5 to 6 yr old) Finn ewes were available for the first part of this study. They were in good body condition and were maintained at the Agricultural Institute, Beclare, Ireland.

During the breeding season (October), estrous cycles were synchronized by a sequence of two injections of 125 μg of a prostaglandin analog, cloprostenol[6], 11 d apart (Acritopoulou et al., 1977); estrus was detected twice daily to identify the 1st d of estrus (d 0). At d 13 following synchronized estrus, all ewes underwent a first laparotomy under general anesthesia, during which all visible follicles were counted and the three largest follicles of each ovary were measured and ink-labeled with dots of India ink in the ovarian stroma surrounding the follicles (Driancourt and Cahill, 1984). All ewes again received 125 μg cloprostenol on d 14 to improve synchrony of corpus luteum regression. At d 15, the three largest follicles of each ovary again were counted, measured and ink-labeled, and those previously marked also were measured. At the third and final laparotomy, about 15 h after beginning of estrus, all marked follicles were measured and all ewes were ovariectomized. Driancourt (1985) showed that the mean deviation of the diameter measured on the ovarian surface in relation to the actual diameter after dissection does not exceed 10%; hence, accuracy of measurement for growing follicles >2.5 mm in diameter is good. Enlarging follicles between successive laparotomies were assumed to be healthy, and regressing ones were assumed to be atretic.

For the second part of this study, 18 T Merino and 11 control Merino ewes were available. They were 7 to 8 yr old, in good body condition and maintained at the CSIRO research farm of Longford Australia, near Armidale.

During the breeding season (early May), estrous cycles of these ewes were synchronized by insertion of intravaginal progestagen sponges (60 mg medroxyprogesterone acetate)[7] for 14 d. Beginning 24 h after sponge removal, ewes were observed every 6 h to determine the beginning of estrus. Ewes were ovariectomized under fluothane anesthesia between 12 and 14 h following the beginning of estrus.

Histological Methods. Immediately after ovariectomy, ovaries were fixed in Bouin Holland's solution, embedded in paraffin and serially sectioned at 10 μm. One of every six sections was mounted, stained with hematoxylin and observed microscopically. All antral follicles (i.e., with cavities exceeding 1,000 μm^2 in their centers) were counted and measured using the oocyte as a marker to avoid counting follicles twice. Follicles were classified as healthy or atretic follicles (i.e., with more than five pyknotic bodies in the section studied). Atretic follicles were ranked in three stages according to the following criteria (Driancourt et al., 1987). Early atretic follicles had 5 to 200 pyknotic bodies, mostly on the border of the antrum, and mitotic figures were sometimes detectable. Advanced atretic follicles had numerous (>200) pyknotic bodies and a thin granulosa cell layer. Late atretic follicles had either no granulosa cells or granulosa cells only around the oocyte. Diameters of follicles were obtained by conversion of the area measured, assuming the follicle was spherical. Healthy and atretic follicles were grouped according to the following six diameter classes (Driancourt et al., 1985): <.35 mm, .35 to .80 mm, .80 to 1.12 mm, 1.12 to 1.59 mm, 1.59 to 2.52 mm, and >2.52 mm.

One randomly chosen ovary of each ewe was counted using this procedure. With the exception of 11 T and 10 control Merino ewes, in which counts of antral follicles were performed in both ovaries, only follicles >1 mm in diameter were studied in the remaining ovaries of all Finn ewes, seven T Merino ewes and one control Merino ewe.

The largest healthy follicles of each ewe, showing a loosening of cumulus cells surrounding the oocyte, were identified as preovulatory follicles. To accurately estimate the number of granulosa cells in preovulatory follicles, the diameter, thickness and density of granulosa cells were measured in the section of each follicle with the greatest circumference,

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[7]Repromap, Upjohn.

and granulosa cell number was obtained by multiplying granulosa cell volume by granulosa cell density. To compare in vivo and histological measures, the amount of shrinkage induced by histological processing was measured on a sample of follicles, which, as part of another experiment, were measured with an ocular graticule just after dissection and again measured after being processed for histological examination. The regression linking follicle diameter after histological processing (y) to actual size after dissection (x) was $y = .90x - .561$ ($r^2 = .96$, n = 157). To allow comparisons between in vivo and histological size, the size after histological processing of the follicles involved in the vivo differentiation of the ovulatory follicles (i.e., those >2 mm in diameter) (Driancourt and Cahill, 1984) had to be identified. This regression was used and showed that 2-mm follicles in vivo measure 1.23 mm after processing. Hence, this 1.23-mm cut-off point was used to define in histological sections the follicles that were involved in vivo in differentiation of the ovulatory follicles.

Statistical Methods. Distributions of follicles were compared by the 2I test (Sokal and Rohlf, 1969); numbers of follicles were compared by one-way ANOVA, or a t-test on raw or log-transformed data when variances were unequal or distributions were not normal. Results are means ± SE.

Results

The number of preovulatory follicles at histological examination was greater (1.9 ± .3, range 1 to 3; $P < .05$) in T Merinos than in control ewes (1.2 ± .1, range 1 to 2). Ovulation rates of high-line Finn ewes measured at d 13 by number of corpora lutea and at d 17 by number of preovulatory follicles (5.4 ± .5 and 5.1 ± .3, respectively) were greater ($P < .05$) than those of low-line ewes (2.7 ± .2 and 3.1 ± .1) and tended to be higher than ovulation rates in control ewes (3.3 ± .6 and 3.6 ± .5, respectively).

One of the high-line ewes had one corpus luteum, two preovulatory follicles and only four antral follicles. This was considered to be an indication of imminent reproductive quiescence, so this ewe was removed from the study.

Follicular Populations. In either T or control Merinos, there were no differences between ovaries in total population of antral follicles (T: 52.1 ± 6.7 and 65.6 ± 9.5 for left and right ovaries, respectively; control 52.7 ± 6.7 and 55.4 ± 9.3, respectively) in their size distribution or in the extent of atresia.

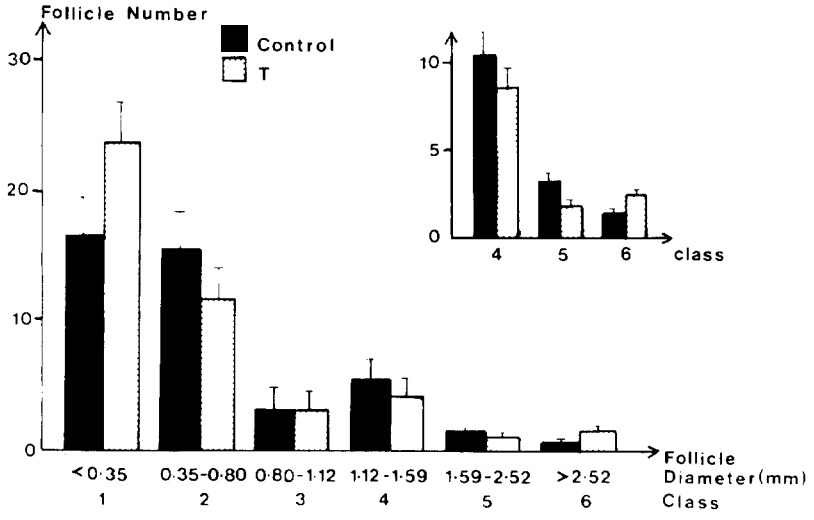

Figure 1. Size distribution of healthy antral follicles of T (n = 11) or control Merinos ewes (n = 18). In the inset, the population of follicles >1.12 mm in diameter of both ovaries of 11 T and 10 Merino ewes is presented.

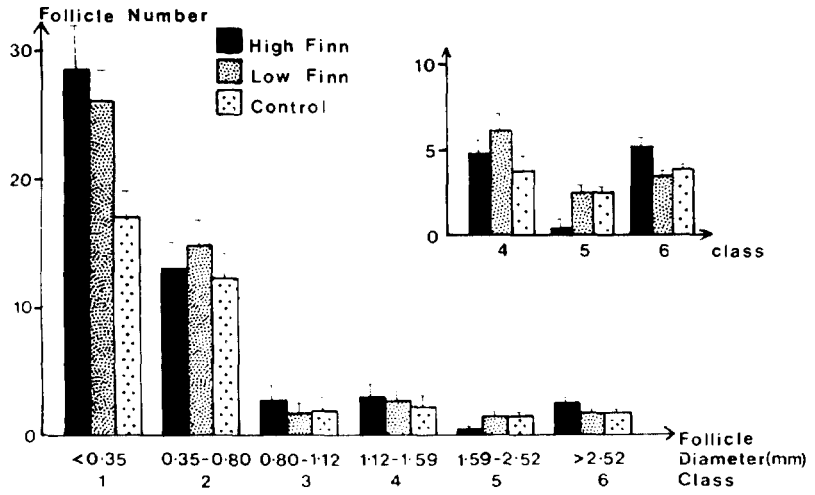

Figure 2. Size distribution of healthy antral follicles of 10 high-, 10 low- and 7 control-line Finns. In the inset, the population of follicles >1.12 mm in diameter of both ovaries of all ewes is presented.

When the follicular populations of the 18 T and 11 control ovaries were compared, the following features emerged. There was no line effect on total number of antral follicles (T: 55.8 ± 4.2 vs control: 52.5 ± 6.2), number of healthy (T: 45.4 ± 3.7 vs control: 42.6 ± 5.7) or atretic antral follicles and ranking of atretic follicles in the different stages of atresia. Distributions of healthy (Figure 1) or atretic follicles in size classes also were similar in the two lines. Furthermore, follicle numbers in the first four size classes (follicles <1.59 mm in diameter) were identical between lines. However, whereas the total number of follicles >1.59 mm in diameter did not differ between lines, T ovaries tended to have fewer follicles in the 1.59- to 2.52-mm size range ($P = .07$) and more follicles >2.52 mm in diameter ($P < .07$). This was confirmed when populations of large follicles of both ovaries of 11 T and 10 control ewes were compared. The T ewes tended to have fewer follicles 1.59 to 2.52 mm in diameter ($P < .06$) and more follicles >2.52 mm in diameter ($P < .01$) than did controls.

Comparison of the follicular population of a single ovary of the three lines of Finn ewes produced similar conclusions. There was no line effect on population of antral follicles (high 56.9 ± 11.0 vs low 53.5 ± 9.0 vs control 42.0 ± 15.4), number of healthy (high 49.4 ± 10.4 vs low 48 ± 7.9 vs control 36 ± 13.6) or atretic antral follicles and ranking of the atretic follicles in the different stages of atresia. Distributions of atretic follicles in size classes did not differ between lines. No line effect was found on size distribution of healthy follicles (Figure 2) or in number of healthy follicles in individual size classes, with the exception of a trend toward a lower number of follicles 1.59 to 2.52 mm in diameter in high-line ewes (.2 ± .1, 1.4 ± .5 and 1.4 ± .8, for high-, low- and control-line ewes, respectively; $P = .1$). These conclusions were further confirmed when follicle numbers of the three largest size classes (i.e., over 1.12 mm in diameter) of both ovaries were compared in all ewes. High-line ewes had significantly more follicles >2.52 mm in diameter ($P < .05$) than did low- and control-line ewes, and they tended to have fewer follicles 1.59 to 2.52 mm in diameter ($P = .1$) compared with the two other lines.

In all three lines, correlations linking the number of antral follicles and ovulation rate were not significant.

Features of the Population of Large Follicles. Analysis of the features (number, size and atresia) of the population of follicles involved in differentiation of the ovulatory follicles (i.e., those larger than 1.23 mm in diameter after histological processing) in both ovaries of T and control Merino ewes showed that size of the largest healthy follicle, excluding preovula-

tory ones, was larger in T than in control Merinos ewes (2.36 vs 1.77 mm, respectively; $P < .05$). None of the other size characteristics differed (Table 1). Regardless of the strain, about half the ewes (6 of 11 in T and 5 of 10 in control ewes) had no large healthy or large atretic follicles >4 mm when preovulatory follicles were excluded.

Overall extent of atresia in the population of follicles >3 mm was reduced in T compared with control ewes (29.4% ± 6.5 vs 57.2% ± 4.5; $P = .02$).

No line difference was found in the diameter of preovulatory follicles (Table 1) or in the number of granulosa cells contained per follicle ($2.1 \times 10^6 \pm .1$ versus $2.3 \times 10^6 \pm .2$ for T and control ewes, respectively; Figure 3).

When characteristics of the population of large follicles were compared in the three lines of Finn ewes, no difference was detected for sizes or numbers of the largest healthy or atretic follicles (Figure 3). Regardless of the line, large healthy (with the exception of preovulatory follicles) or atretic follicles >4 mm were seldom found. The overall extent of atresia in the population of follicles >3 mm was identical between lines (14.1 ± 5.7%, 12.2 ± 6.6% and 10.6 ± 5.8% for high, low and control ewes). In contrast, the diameter of preovulatory follicles differed between lines (Table 1). High-line follicles contained fewer granulosa cells (Figure 3; $.96 \times 10^6 \pm .04$) than low ($1.46 \times 10^6 \pm .07$; $P < .05$) and control-line follicles ($1.39 \times 10^6 \pm .10$; $P < .05$). However, total number of granulosa cells of ovulatory follicles was similar in the three lines (high, $4.4 \times 10^6 \pm .41$; low, $4.4 \times 10^6 \pm .20$; control, $4.7 \times 10^6 \pm .23$). Furthermore, granulosa cell content and number of preovulatory follicles were correlated in control ewes ($r = -.79$; $P = .02$).

Kinetics of Differentiation of the Ovulatory Follicles in Finns. One day before luteolysis, no difference was found between lines in the number of large (>5 mm), medium + large (>3 mm in diameter) or total number of visible follicles (>1 mm in diameter; Table 2). Between the first two laparotomies, the number of follicles growing to reach at least 3 mm ("recruited follicles") tended ($P < .1$) to be greater in the high-line than in the low-line or control ewes (Table 2). Among these recruited follicles, the rate of loss through regression was low and similar between lines (Table 2). Growth rate of ovulatory follicles either between d 13 and 15 or between d 15 and 17 was unaffected by genetic line. Of the ovulatory follicles, 5.3, 5.3 and 8.5% were never ink labeled in high, low and control ewes, respectively; 46, 56 and 78% of the ovulatory follicles of high, low and control ewes were already labeled at d 13 (high vs control, $P < .1$).

Discussion

The main conclusions of this study were 1) that the number of antral follicles, the extent of atresia and the size distribution of healthy and atretic follicles in ewes selected for high ovulation rate and their controls were similar; 2) that only terminal follicular growth and differentiation were affected to generate an increased ovulation rate in T Merinos and high-line Finns; and 3) that selection for high ovulation rate in Finns and Merinos altered the processes of differentiation of the ovulatory follicles in different ways.

The first conclusion is in agreement with a number of previous reports about sheep. The reports demonstrated that between breeds (Lahlou-Kassi and Mariana, 1984; Driancourt et al., 1986) or strains (Driancourt et al., 1985; McNatty et al., 1986) or within breeds (Driancourt and Fry, 1988), differences in ovulation rate from two- to fourfold were associated with no or limited differences in overall number of antral follicles. This conclusion is supported further by the lack of correlation between the number of antral follicles and ovulation rate in this study using prolific T and high Finn ewes. The lack of relationship between ovulation rate and the amount of growing follicles also has been demonstrated when prolific sows from Nebraska selection lines were compared with controls (Kelly et al., 1988).

The second conclusion confirms earlier findings in prolific breeds of sheep (Driancourt et al., 1985, 1986). Comparative histological study of ovaries of T and control Merinos demonstrated that alterations in the extent of atresia but not in size or number of granulosa cells per follicle were associated with increased ovulation rate of T ewes. Factors that induce a larger size of the largest healthy follicle and a reduced amount of atresia among follicles >3 mm in diameter in T ewes are unknown.

In contrast, the superiority of the ovulation rate of high-line Finn ewes over the other lines was related to changes in number and size of

TABLE 1. HISTOLOGICAL FEATURES OF THE POPULATION OF FOLLICLES >1.23 MM IN DIAMETER IN THE TWO LINES OF MERINO AND THE THREE LINES OF FINNS

	Size of preovulatory follicles, mm	Size of largest healthy follicles following preovulatory ones, mm	Size of largest atretic follicle, mm	Mean size of three largest healthy follicles following the preovulatory ones, mm	Mean size of three largest atretic follicles, mm
Finn ewe line					
High Finn	4.19[a] ± .06 (10)[c]	1.84 ± .03 (9)	2.30 ± .05 (10)	1.53 ± .03 (7)	2.14 ± .05 (7)
Low Finn	4.98[b] ± .09 (10)	1.83 ± .04 (10)	2.54 ± .06 (10)	1.74 ± .03 (9)	2.21 ± .06 (9)
Control Finn	4.47[a] ± .08 (7)	2.11 ± .09 (6)	2.32 ± .09 (6)	1.84 ± .05 (4)	1.99 ± .06 (5)
Merino line					
T	4.73 ± .10 (11)	2.36[a] ± .07 (11)	3.16 ± .09 (11)	1.95 ± .03 (11)	2.47 ± .04 (11)
Control	4.80 ± .09 (10)	1.77[b] ± .04 (10)	3.41 ± .12 (10)	1.73 ± .06 (8)	2.68 ± .04 (10)

[ab]Means within a column and breed line with a similar superscript are not different ($P < .05$).

[c]Number of ewes represented in the value.

TABLE 2. COMPARATIVE FEATURES OF PREOVULATORY DIFFERENTIATION OF LARGE FOLLICLES IN THE THREE LINES OF FINN EWES MEASURED AT REPEATED LAPAROTOMIES (MEAN ± SE)

	Number of follicles on the ovaries at d 13			Number of follicles recruited between d 13 and d 15	Percentage of these follicles reaching ovulation	Growth rate of the ovulatory follicles	
	>5 mm	>3 mm	Total			13–15 mm	15–17 mm
High (n = 10)	1.3 ± .5	5.6 ± .6	17.8 ± 2.7	5.4 ± .6[a]	87.5 ± 5.6	.66 ± .09	.63 ± .05
Low (n = 10)	2.1 ± .3	6.2 ± .6	19.2 ± 2.2	4.2 ± .4[b]	82.7 ± 6.5	.56 ± .07	.87 ± .11
Control (n = 7)	1.8 ± .5	5.7 ± .3	13.3 ± 2.9	4.4 ± .3[b]	78.5 ± 8.7	.80 ± .09	.57 ± .11

[a,b]Means within a column with a similar superscript are not different ($P < .10$).

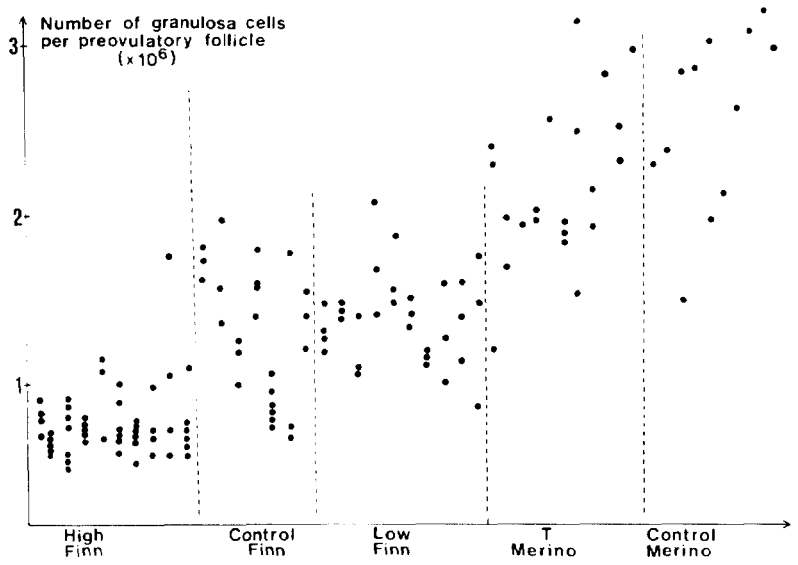

Figure 3. Number of granulosa cells per preovulatory follicle in individual follicles of ewes of the three Finn lines and T and control Merinos (each vertical line corresponds to one ewe; each dot is a follicle).

recruited follicles as well as in size of the preovulatory follicles and in their cell numbers, not to alterations in the diameter of the largest healthy or atretic follicles. That reduced preovulatory diameter is associated with high ovulation rate has been demonstrated previously in a wide range of prolific breeds (Finn: Webb and Gauld, 1985; Romanov: Driancourt et al., 1986; Booroola: Driancourt et al., 1985; McNatty et al., 1986). Reduced numbers of granulosa cells per preovulatory follicle have been found in some (Finn, Booroola) but not all (Romanov) prolific breeds (McNatty et al., 1986; Driancourt and Fry, 1988). Factors mediating these changes in the number of recruited follicles and in preovulatory follicle size and granulosa cell content and hence contributing to high ovulation rate of the high-line Finns have not been fully clarified. Differences in FSH concentrations between high-line Finns and control Finns were not detectable (Adams et al., 1988). Hence, one of the other regulatory steps of the control of ovulation rate presumably was affected by selection for high ovulation rate in Finns. An altered follicular sensitivity to gonadotropins (Henderson et al., 1985), altered local (autocrine and paracrine) regulations (Bindon et al., 1986; Driancourt and Fry, 1988) might explain the alterations of follicular development found in high-line Finn ewes.

Follicle-stimulating hormone concentrations previously were shown to be fairly heritable (Bodin et al., 1986). Because the increase of ovulation rate in high-line Finns is not associated with increased FSH secretion, we conclude that the high heritability of ovulation rate is due to other determinants of ovulation rate. This suggests that these traits also are highly heritable.

Implications

Selection for altered ovulation rate in Finn and Merino ewes has been successful. Although antral follicle numbers were not affected by selection, altered ovulation rate was related to changes in growth and differentiation of the ovulatory follicles. These changes differed between Merino and Finn ewes. The control mechanisms altered are still unknown. However, because selection was efficient, heritability of these control mechanisms must be high.

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