THE INCIDENCE OF MORPHOLOGICALLY ABNORMAL OVA IN SHEEP

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[*Manuscript received October* 25, 1963]

Summary

The incidence of morphologically abnormal, unfertilized eggs in aged Merino and Merino cross ewes was studied over 15 months. The overall incidence was 13·8%; there was no significant seasonal variation in incidence.

However, the incidence increased significantly with increasing post-ovulatory age up to 3–4 days. It was concluded that most of the abnormalities represent degenerative changes such as might be expected in aging eggs.

I. Introduction

The reports of Dutt (1954), Hart (1956), and Laffey and Hart (1959) indicate that the incidence of gross morphological abnormalities in sheep ova may be as high as 20–40% at certain times in the breeding season. In some instances this appears to be related to high environmental temperatures just prior to ovulation; a significant increase in the incidence of abnormal ova has been obtained by exposing ewes to raised environmental temperatures (Dutt, Ellington, and Carlton 1959; Alliston, Egli, and Ulberg 1961).

It was of interest, therefore, to examine the incidence of abnormal ova under Australian conditions. This has been done using material from aged unmated ewes that were being slaughtered at a commercial abattoir.

II. Materials and Methods

Each week from December 1, 1961, to March 28, 1962, 40–100 genital tracts from aged non-pregnant ewes were collected from a nearby abattoir. The ewes were mainly strains of Merino, or Merino × Corriedale, or Merino × Border Leicester crosses; they came from the Central- and Southern-western Slopes of New South Wales. The tracts were placed in polythene bags, allowed to cool to ambient temperature (about 20°C), and brought back to the laboratory. Tracts with ovaries containing corpora lutea estimated as 0–3 days old were selected and their fallopian tubes flushed with 0·9% saline. The eggs were placed on a slide in saline and a cover-slip supported with grease on the four edges was placed over them. Phase-contrast microscopy was tried but, because of the highly refractile granules usually present in sheep eggs, narrow-aperture illumination, which enabled details to be seen better, was generally used. The eggs were recovered and examined within 2–6 hr after slaughter of the ewes.

Between April and June 1962 and between October 1962 and March 1963, 18 more collections of genital tracts were made. The sheep were similar to those in

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the first series of observations and were from the same geographical areas. In this series tracts were collected only from ewes that had been marked by vasectomized rams fitted with Sire-Sine ewe-marking harnesses. Five rams were joined with about 200 ewes 4 days before slaughter and each morning the ewes with fresh marks were identified.

III. Results

In the period December 1961 to March 1962, a total of 233 eggs was recovered from the fallopian tubes of 208 ewes. Of these 17 were fertilized; they were in various stages of development from the pronuclear to the 12-cell stage. Of the 216 unfertilized eggs, 26 (12·0%) were classified as abnormal. The variation between months was not significant: for successive months the incidence of abnormal eggs was 5 out of 47 (10·6%), 9 out of 66 (13·6%), 7 out of 40 (17·5%), and 5 out of 63 (7·9%).

Table 1

Incidence of abnormal eggs in ewes slaughtered 0–4 days after marking by vasectomized rams

Unfertilized Eggs Recovered	Interval between Marking and Slaughter			
	0–1 Day	1–2 Days	2–3 Days	3–4 Days
From uterus and tubes				
Total	13	59	76	117
Abnormal	0	4	16	34
From tubes only				
Total	13	58	72	90
Abnormal	0	3	14	19

The types of abnormality encountered were:

(1) broken zona pellucida—3 eggs;
(2) vitellus of irregular shape (cf. Plate 1, Figs. 1 and 2)—9 eggs;
(3) vitellus ovoid in shape and containing granule-free area(s) (Plate 1, Fig. 3) or surface blebs—4 eggs;
(4) the perivitelline space rather large and containing a number of clear spheres or cylinders [such as depicted in Figure 3 of Hart (1956) and in Plate 2, Figure 2, of Averill (1958)]—7 eggs;
(5) fragmented vitellus—1 egg;
(6) vitelline membrane apparently broken down—1 egg;
(7) vitellus containing a large granule-free area—1 egg.

It seemed likely that many of these abnormalities were an expression of normal degenerative changes in unfertilized eggs. If this is so, an increase in the incidence of abnormalities would be expected with increasing time after ovulation. Accordingly, in the second series of observations (April 1962 to March 1963) only tracts from ewes that had been marked by the harnessed vasectomized rams 0–4 days before slaughter were flushed for eggs. The results are summarized in Table 1. After combining the results for the intervals of 0–1 and 1–2 days (in most instances ovulation had not

yet taken place in ewes marked in the 24 hr before slaughter) a χ^2 test indicated significant heterogeneity ($P < 0{\cdot}001$) between the results from ewes examined 3–4 days (29·1% of eggs abnormal), 2–3 days (21·1%), and 0–2 days (5·6%) after being marked by the vasectomized rams. Significant heterogeneity was still present after exclusion of eggs that had been recovered from the uterus.

There was again no evidence of seasonal variation in the incidence of abnormal eggs (Table 2). The overall incidence of abnormal eggs recovered from the fallopian tubes was 15·5%; this was not significantly higher than the incidence found in the first series. The tracts from the last three collections (listed as March, Table 2) were stored at 4°C for 20 hr before recovery of the eggs, but no increase in the incidence of abnormal eggs was detected.

TABLE 2

ABSENCE OF SIGNIFICANT SEASONAL VARIATION IN THE INCIDENCE OF ABNORMAL EGGS

Second series of observations

Collection Period	Incidence of Abnormal Eggs as Proportion of:	
	All Eggs Recovered	Eggs Recovered from Fallopian Tubes Only
April–June, 1962	23 out of 126 (18·3%)*	19 out of 114 (16·7%)†
October–November, 1962	8 out of 33 (24·2%)*	5 out of 29 (17·2%)†
January–February, 1963	15 out of 64 (23·4%)*	6 out of 50 (12·0%)†
March 1963	7 out of 41 (17·1%)*	6 out of 40 (15·0%)†

* $\chi^2_3 = 1{\cdot}27$. † $\chi^2_3 = 0{\cdot}67$.

In sheep the eggs normally take 2½–3½ days to traverse the tubes and enter the uterus (Clark 1934; Kelly 1937; Edgar 1962). In some instances in the present study, egg transport was apparently rapid. Four eggs were recovered from the uteri of ewes examined 2–3 days after marking, and in one ewe slaughtered 1–2 days after marking an egg with an intact corona radiata and large cumulus oophorus was recovered from the uterus. In sheep, dissolution of the cumulus and corona normally takes place soon after ovulation (Clark 1934; Pitkianen 1958). It was uncommon to find eggs with these layers still present: they were present in only 5 out of a total of 72 eggs recovered from ewes slaughtered 0–2 days after marking. In the first series of observations two eggs in a continuous mass of cumulus were recovered from the tube of one ewe (Plate 1, Fig. 4).

Using an eyepiece micrometer, measurements were made on the eggs soon after recovery. There was no significant difference between normal and abnormal unfertilized eggs in vitelline or overall diameter, nor was there much change with increasing time after ovulation, except that, in eggs recovered from the tubes, the diameter of

the zona tended to increase (Table 3). The increase in the mean volume of the perivitelline space in the 3 days after ovulation (i.e. between the 0–1-day and 3–4-day results) was 17%. Eggs recovered from the uterus tended to be smaller than those from the tubes.

Of the four abnormal eggs recovered 1–2 days after marking, three were of the second and one of the fourth above-listed types.

TABLE 3

DIMENSIONS OF MORPHOLOGICALLY NORMAL EGGS RECOVERED FROM THE UTERI OR TUBES OF EWES 0–4 DAYS AFTER MARKING

Mean ± standard error of mean given

Interval between Marking and Slaughter (days)	Diameter of Vitellus (μ)	Overall Diameter (μ)	No. of Eggs Measured
	Eggs from Fallopian Tubes		
0–1	126±1·4	167±2·1	12
1–2	130±0·9	168±1·2	48
2–3	128±0·9	173±1·0	54
3–4	126±1·1	176±1·3	61
	Eggs from Uterus		
2–4	110±1·6	169±2·4	13

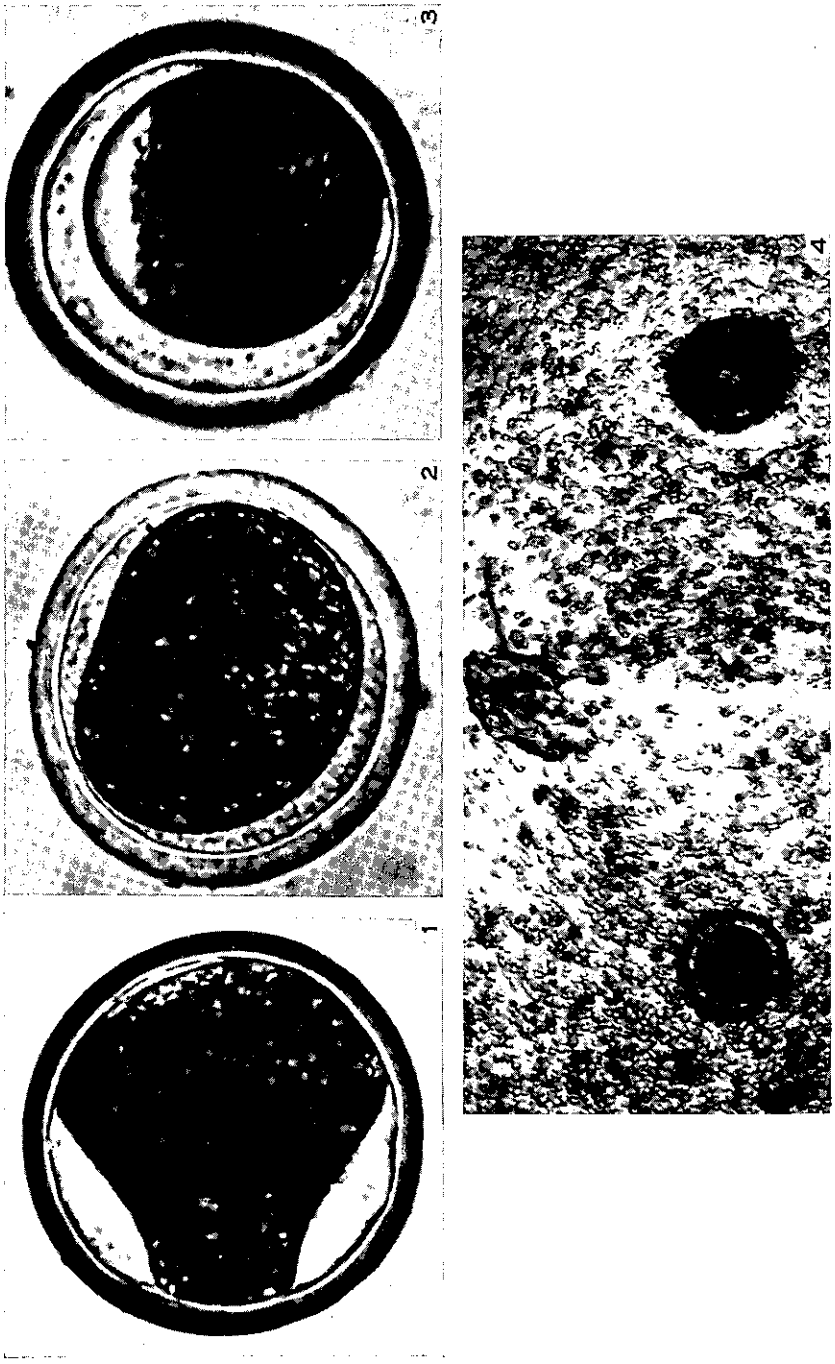
IV. DISCUSSION

The present investigation has given no indication of a marked seasonal variation in the incidence of abnormal eggs from fallopian tubes in sheep, such as has been reported from New Zealand (Hart 1956; Laffey and Hart 1959). However, the ewes investigated were mainly Merino or Merino crosses, and these breeds generally show less seasonal fluctuation in reproductive performance than the "Corriedale type" ewes studied by Hart.

The classification of an egg as abnormal was based on obvious morphological deviations. The observations of Hancock and Hovell (1961) in sheep indicate that some fertilized eggs that appear morphologically abnormal are nevertheless capable of normal development. On the other hand, most of the eggs classified as normal in the present study would have been incapable of normal development, having passed the end of their fertile life (10–18 hr after ovulation—Dauzier and Wintenberger 1952; Moule and Braden, unpublished observations), and therefore, strictly speaking, were "abnormal". In other words, the final criterion of normality must be the ability to develop normally, and this of course, depends on the participation of a spermatozoon.

In the present study, as in earlier ones (Dutt 1954; Averill 1958; Laffey and Hart 1959), eggs with a cracked or broken zona pellucida were classified as abnormal. However, as recovery of the eggs from the fallopian tubes entails flushing with saline under pressure, it is not unlikely that some zonae are broken in the process. Eggs

ABNORMAL OVA IN SHEEP



Figures 1–4 are of fresh unfixed eggs photographed under narrow-aperture illumination: *1–3*, types of abnormal unfertilized eggs, ×320; *4*, two recently ovulated sheep eggs recovered from a fallopian tube with intact and confluent cumuli oophori, ×75.

may vary in their sensitivity to mechanical rupture, and this sensitivity may be increased under certain conditions [e.g. with increasing time after ovulation or in ewes subjected to high environmental temperatures (Dutt, Ellington, and Carlton 1959; Alliston, Egli, and Ulberg 1961)], but, nevertheless, if left *in situ*, the defect may not prevent normal development, and therefore the eggs should not be classified as abnormal.

The actual incidence of abnormal eggs found in the present study was relatively low and probably of little significance as a cause of the poor reproductive performance of sheep in Australia (Moule 1960). Furthermore, it would seem that most of the abnormalities developed 1–3 days after ovulation and represent degenerative changes that might well be expected in aging unfertilized eggs.

V. Acknowledgments

Thanks are due to the Riverstone Meat Co. Pty. Ltd., in particular to Mr. N. J. Bargwanna and Mr. G. Bloxsom, for the use of facilities at Riverstone abattoir and for assisting us in the collection of material from slaughtered sheep.

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