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# Sources of Loss in the Unfertilised and Fertilised Sheep's Ova

By D. S. HART, Canterbury Agricultural College, Lincoln.

**M**ANY and varied have been the methods of approach adopted by scientists towards the problem of fertility in livestock. Each one has made some contribution to the overall picture with the result that to-day we appear to have nearly completed our knowledge of the highly complicated physiological processes involved in reproduction.

There are, however, one or two aspects of reproduction which still await a full explanation and it is the introduction to one of these which I propose to discuss to-day.

A large amount of the investigational work has hitherto been concentrated on the male's contribution to the fertility problem and this to such good purpose that we can now handle sperm without fear of damage, and for almost an indefinite period of time with no loss of fertility; yet on the other hand, the knowledge of the ovum is most limited—we have yet to develop techniques which will enable us to handle ova for more than a few hours without some impairment of their fertility. Also, for the present we do not seem to be able to produce, under laboratory conditions, the natural uterine environment necessary for fertilisation, or prolonged incubation of the fertilised ovum. Until our present techniques are improved on these two vital points to a degree comparable with our sperm procedures, the rate of progress will continue to be slow.

The relative lack of detailed knowledge about the female's contribution during the ovum production, fertilisation and early development stages of reproduction appears to have caused a greater concentration of effort towards an understanding of the male's contribution. This has resulted, perhaps, in over-much emphasis being placed on the male and resulted in his taking the major blame which is regarded as his privilege, for any infertility which may occur.

It is in an endeavour to discover to what degree the female should be blamed for infertility that I have risen to the defence of the male in this paper.

## Materials and Methods:

In each breeding season since 1950 all ewes killed in the Lincoln College slaughter house have been used to provide information and experimental material. Immediately upon slaughter the complete reproductive organs of each ewe were removed and taken to the laboratory. The ovaries were examined with respect to the follicular or corpora lutea development relative to the known pre-slaughter breeding behaviour and treatment. Tubal ova were recovered by dissecting the oviducts from the mesosalpinx, severing the tube about the upper third of the cornu of the uterus. The dissected oviducts were then flushed from the fimbriated end to the cornu with either physiological saline or homologous serum at 37 degrees C. into a watch-glass. The flushings were then searched until the ova were located and they were then transferred to a hanging drop slide for photography and further examination under the microscope.

During all microscopical examinations an endeavour was made to keep the temperatures of the ova at 37 degrees C. by means of elec-

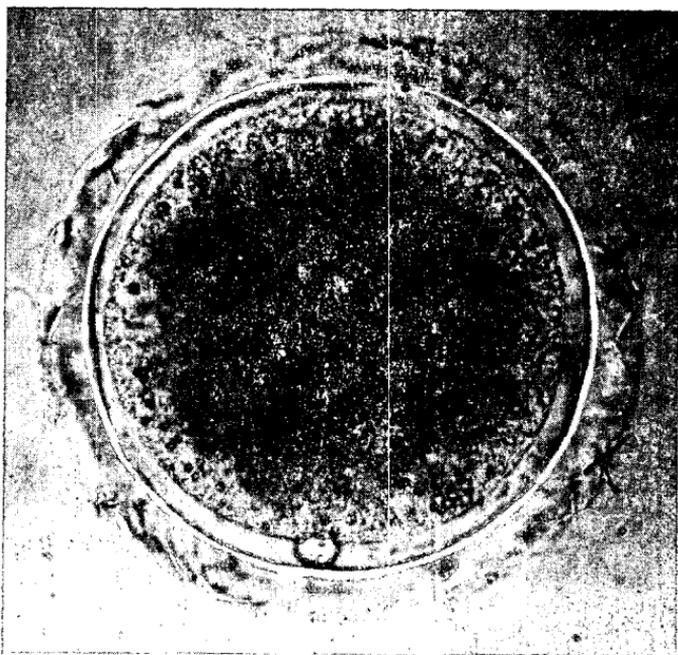


Fig. I.

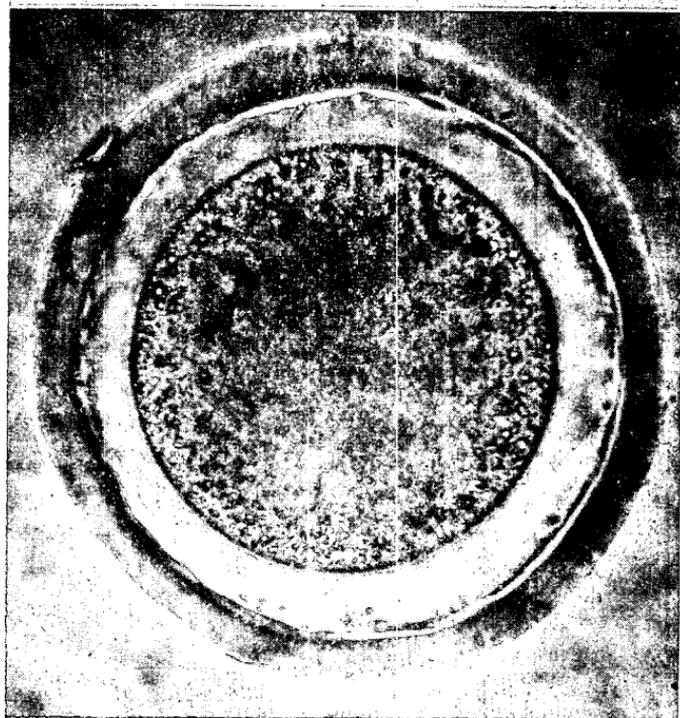


Fig. II.

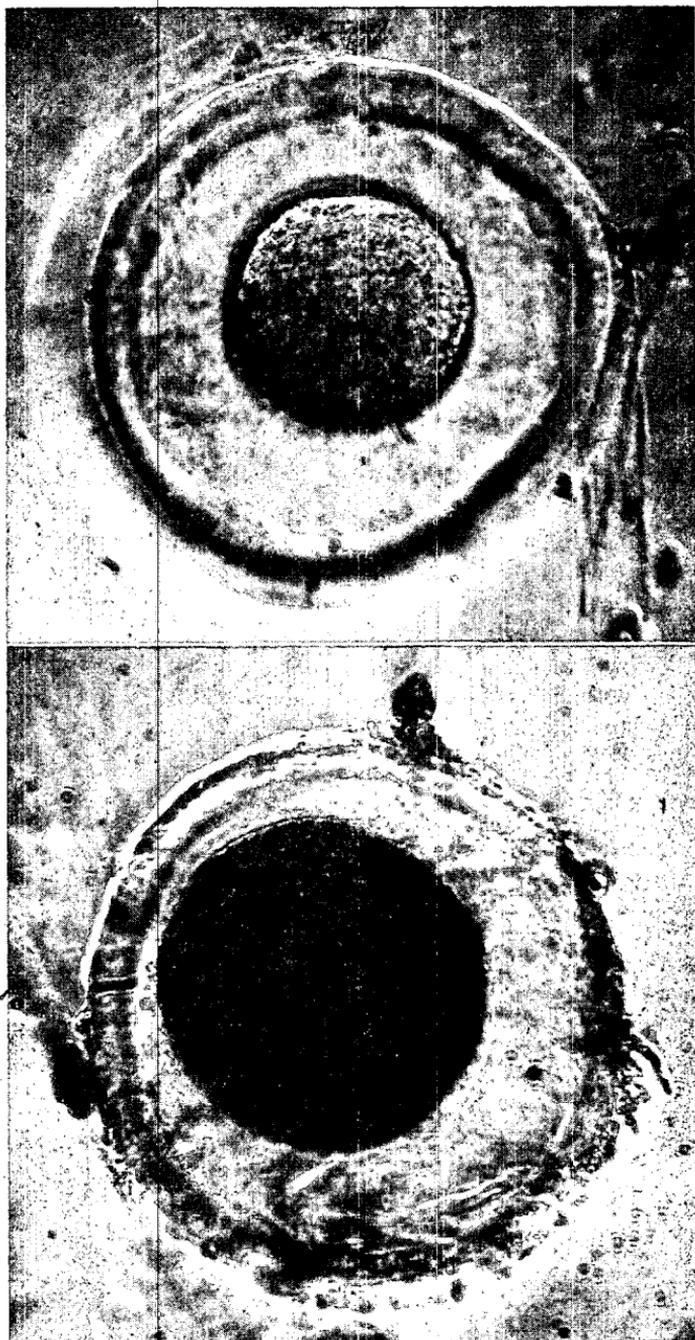


Fig. II.

trically heated slide holders and the use of an incubator for storing all fluids and instruments in immediate use.

Uterine ova were recovered in a similar manner except that the larger amount of flushing fluid required necessitated collection of the flushings in small petri dishes.

In all cases where fertile matings had been made prior to slaughter, sperm smears were taken in the uterus, cornua and oviducts in order to determine whether any particular ovum had every opportunity, from the male point of view, of being fertilised.

Rams run with the killing mob were kept ocred on the brisket and a close watch was kept on their sperm quality, and reproduction throughout the trials. A detailed examination was made of the reproductive organs of all ewes after completion of the ova recovery technique for any anatomical abnormality which might interfere with the fertilising of the ovum. This included measurements of cervical length. Unfortunately it will not be possible to discuss all these results in this paper.

During the six-year period of this trial a total of 866 ewes have been examined, producing over 1000 ova for the investigation.

### Results and Discussion:

As there appeared to be no literature covering what was a normal or an abnormal sheep's ovum, the first five years of the experiment were largely devoted to an attempt to define these terms in some measure.

It will be appreciated that it is almost impossible to arrive at a satisfactory method or criterion for determining this with our present limitations in ova culture. The method used in this investigation has been the "fertilisability test," defined as follows:—

A single cell ovum was deemed to be normal, up to its present stage of development, if it was fertilised or able to be fertilised, when recovered. Naturally a considerable proportion of this defining had to be done on a comparative basis. It was accepted that all dividing ova recovered had been and were normal up to their present stage of development unless obvious signs of abnormalities were then appearing.

From the experience gained in this investigation it would appear that a fertilised sheep's ovum may always be identified by the numerous surplus sperms which can be found adhering to, or embedded in the Zona pellucida. This meant that with the sheep ova the Evans blue absorption method used by Brock and Rowson (1952), to determine whether fertilised or not, could be discarded.

With experience and the acquisition of a new microscope, we can now identify fertilised single cell ova by certain changes taking place in the nucleus, and it is hoped that this will be further improved upon shortly by identification of the male and female pronuclei within the nucleus on similar lines to Blandau's (1952) results with rat ova.

Fig. I shows a single cell fertilised ovum in which the points already detailed may be clearly seen: (a) the surplus sperms adhering to and partially embedded in the zona pellucida; (b) the organisation taking place in the nucleus immediately prior to the first division; (c) the obvious lack of any anatomical abnormality in ovum structure.

In addition I would draw your attention to one of two polar bodies which were extruded in this egg.

### Normal Ova:

The first and most difficult stage of this investigation—the ability to define what is a normal ovum—was now able to be completed. After systematically examining several hundred ova and considering the following basic measurements:

1. Ovum diameter.
2. Zona pellucida thickness.
3. Perivitelline space.
4. Nucleus diameter.
5. Nucleus orientation.
6. Nucleus stability.

we are at present classifying normal ova into three basic types—A, B and C. Examples of these are shown in Fig. II and it will be seen that there is considerable variation between what we now know to be normal ova.

### Abnormal Ova (Single Cell):

In compiling the classification of abnormal ova it should be remembered that no single cell ovum was designated abnormal unless there was proof from sperm smears that it had been in contact with sperm in the oviduct and could be presumed to have had every opportunity of becoming fertilised.

To date we have been able to classify two distinct types of abnormalities:

1. Ova showing involution abnormality,
2. Ova showing vacuolation abnormality.

Fig. III shows typical examples of these two types.

The third group of abnormalities contains all those which have not yet been classified but it consists in the main of ova which show some gross abnormality of the nucleus and an example of these is shown in Fig. IV.

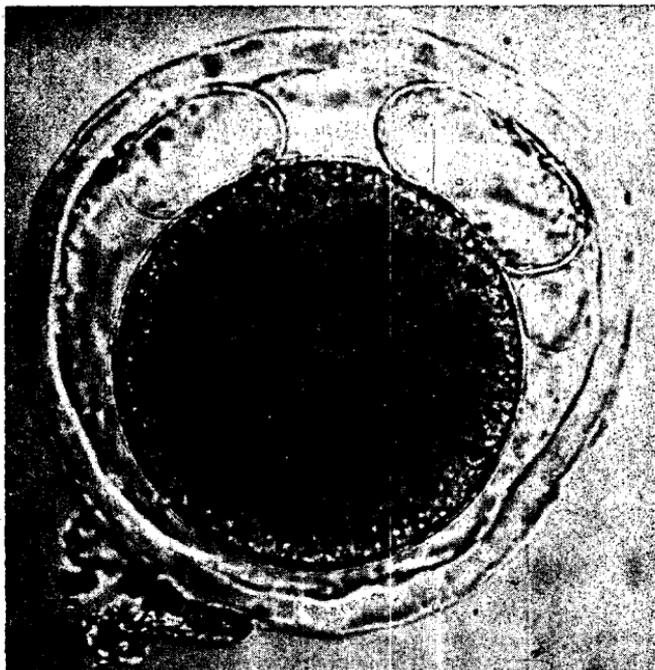
It should be pointed out that single cell ova showing any of the above mentioned abnormalities will never be fertilisable.

### Abnormal Ova (Fertilised):

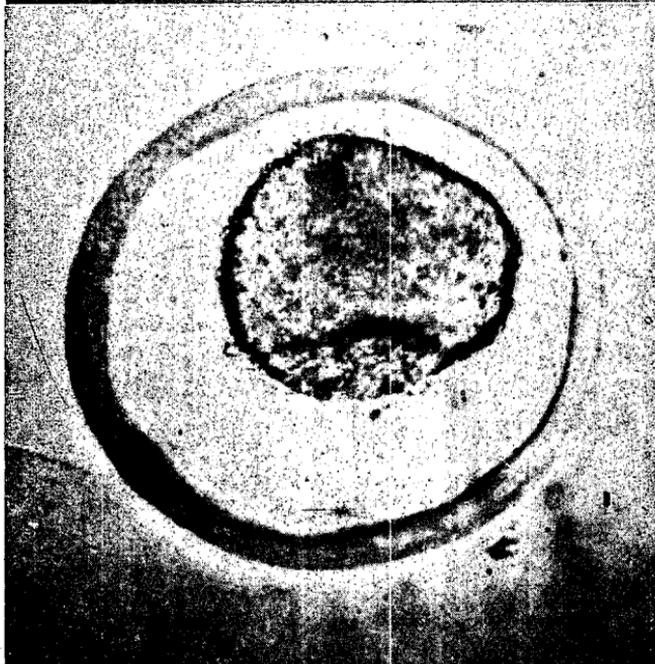
This section provides an interesting study as it covers the development of the ovum in two different environments—the oviduct and the uterus, but for the purposes of this paper it will be necessary to confine ourselves to the one type of abnormality which appears to be able to develop subsequently to fertilisation and division.

Vacuolation, we have found, can also occur in the already fertilised and dividing egg and finally bring about the death of that egg. It is not possible at this stage to determine whether the condition already existed in the single cell ovum prior to fertilisation, and had not developed sufficiently to prevent fertilisation, as mentioned previously, or that the condition had arisen post-fertilisation. In either case the result is the same, the complete loss of that ovum for production purposes. This condition is illustrated in Fig. V. The hypothesis of the growth of vacuolation is clearly demonstrated by Fig. VI which shows an otherwise normal cell egg which when first recovered showed the very slightest evidence of incipient vacuolation. This egg was incubated for 24 hours and then re-photographed, clearly showing the growth of vacuolation during that period.

In what appears to be the first report mentioning abnormal ova, Dutt (1954) presents photographs of two ova which definitely appear to be showing vacuolation, although he calls one a shrunken ovum and the other a fertilised degenerating ovum. He offers no comments on his abnormal ova other than the percentage; nor does



Vacuolated ovum.



Involutated ovum.

Fig. III.

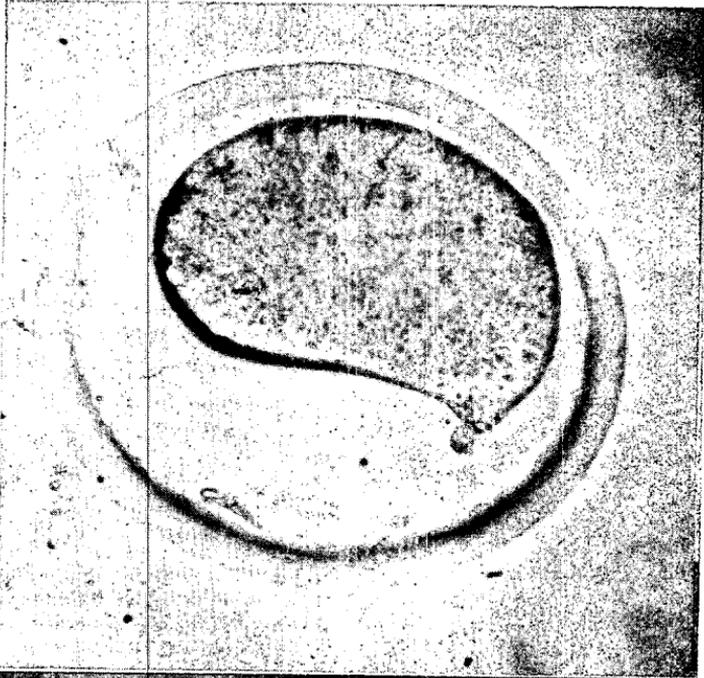


Fig. IV.

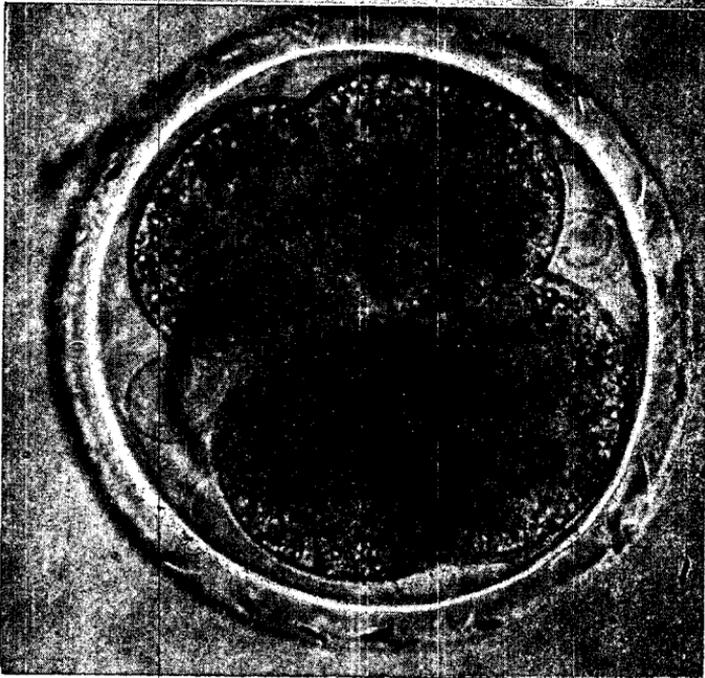
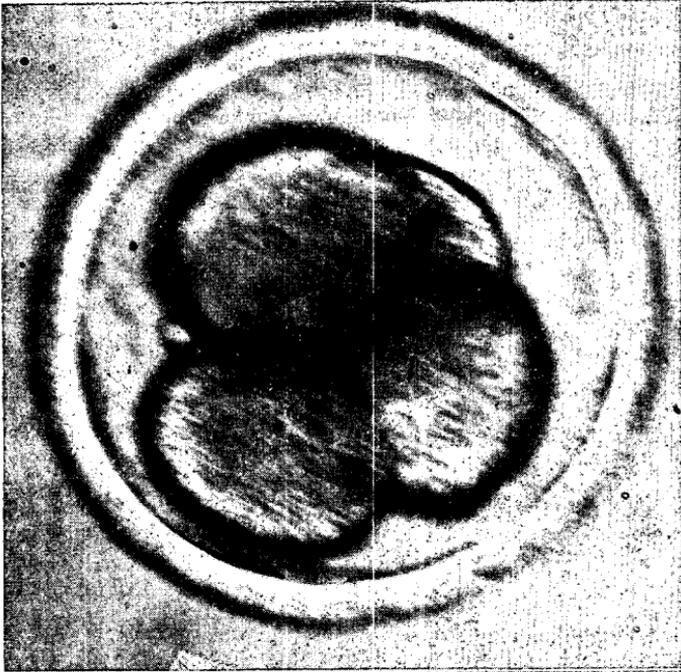
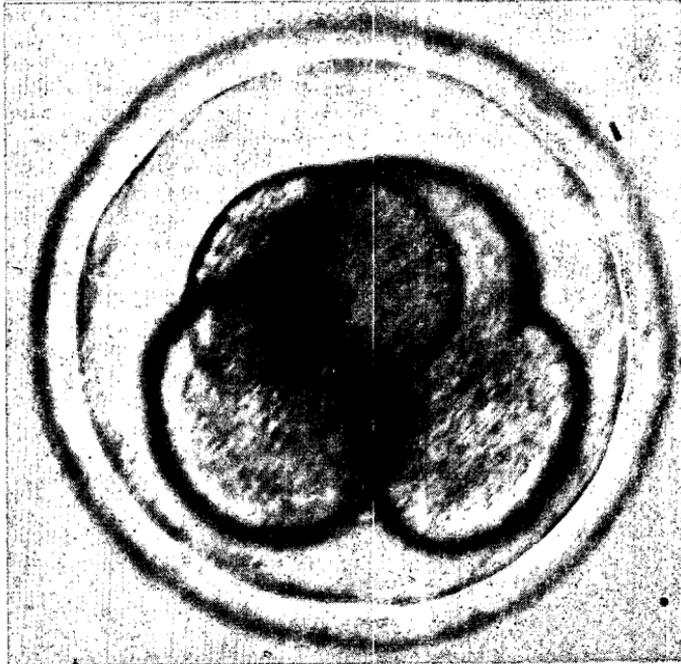


Fig. V.



After Incubation.



Before Incubation.

Fig. VI.



Fig. VII.

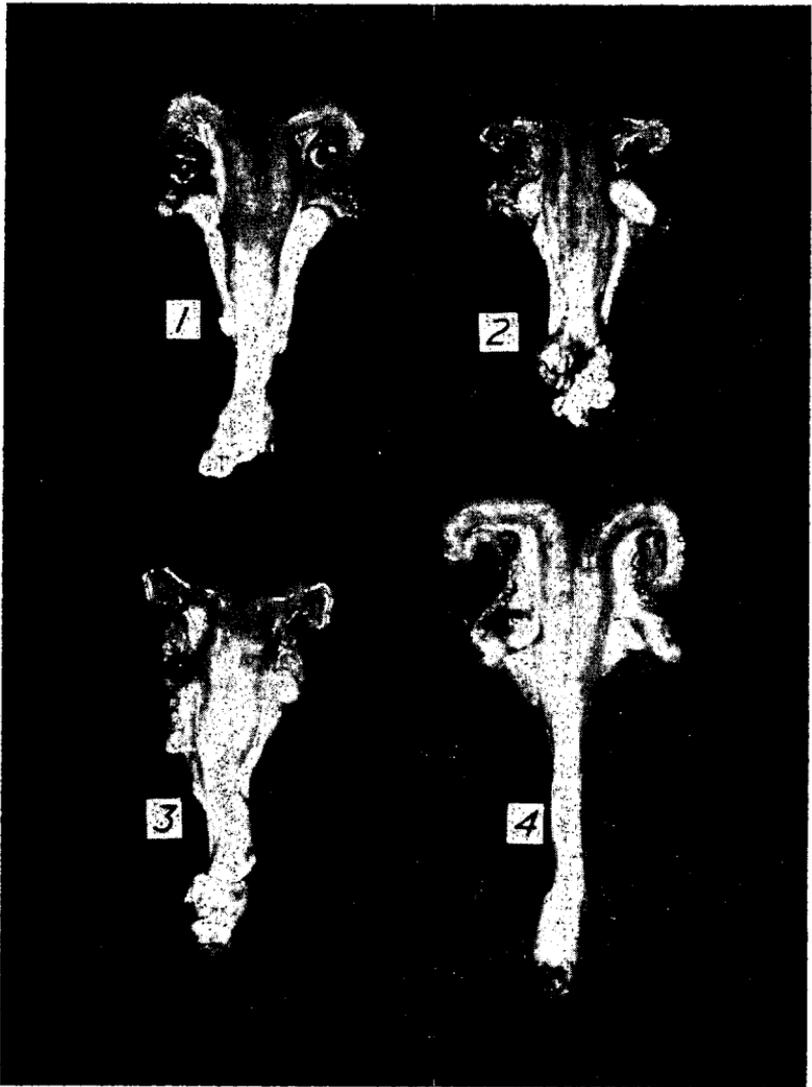


Fig. VIII.

he comment on what he has called normal ova; in fact, from the photograph of what he terms a normal two-cell ovum I would say there appears to be vacuolation of about the same magnitude as shown in Fig. VI.

At this point I would remind you that these ovum abnormalities and obvious losses cover only a small section of the total reproductive period, namely, the period between ovulation (say oestrus) and primary implantation (say 8-11 days) in the ewe, also that they are the sole responsibility of the ewe, and the ram must be regarded as free from blame for this situation.

Tables I and II show a factual analysis of the situation as far as it affects this particular period.

**TABLE I**  
**1955 Breeding Season Results**

	Total	Percentage
Ewes examined .....	252	
Ewes ovulating .....	210	83.3
Number of ovulations .....	295	
Average ovulations per ewe .....	1.40	
Ova recovered .....	220	74.6
Normal ova .....	182	82.7
Abnormal ova .....	38	17.3

**TABLE II**  
**1955 Breeding Season**  
**Classification of Abnormal Ova**

1. Involution .....	24	63.2%
2. Vacuolation .....	5	13.2%
3. Unclassified .....	9	23.6%
Total .....	38	100.0

It will be noticed that the ovulation rate per ewe over the breeding season of 1.4 in this sample agrees closely with the 1.47 of Dutt (1954). But although our sample of basically Corriedale type ewes would be only regarded as medium in fertility, the ovulation rate is much higher in the early part of the breeding season, being 1.53 in April. Now with a lambing percentage of 100 this means with these sheep over 30% of eggs shed will never become live lambs. The production of 17% of abnormal ova largely covering losses up to the primary implantation period alone, seems a substantial contribution from the ewe towards infertility and I hazard a guess that many a good ram has had to accept the blame for this in the past.

It is interesting to note that involution is responsible for half of the abnormal ova produced and that it only affects single cell eggs, preventing fertilisation. Vacuolation, on the other hand, can prevent fertilisation, or destroy the fertilised egg prior to primary implantation.

But the ewe still has another peak period where losses occur. This is around 23rd day of pregnancy, or the secondary implantation stage. The reasons for this loss are also as obscure at present as the cause of the ova losses. The fact that one of twin embryos, both existing under the same uterine environment in respect of nutrition, hormone balance and all other factors, should suddenly die seems inexplicable at this stage. Fig. VII shows twin embryos recovered from the same uterus, one alive, the other dying.

### Length of Cervix:

In the course of this investigation the length of all cervixes from mature ewes was measured and it is thought that the enormous range in length, as shown in Figs. VIII and IX may perhaps be playing some part in diluting and delaying the sperm during their progress towards one of the 82.7% eggs which are fertilisable. This then may prove to be one further contribution of the ewe towards infertility not attributable to the ram.

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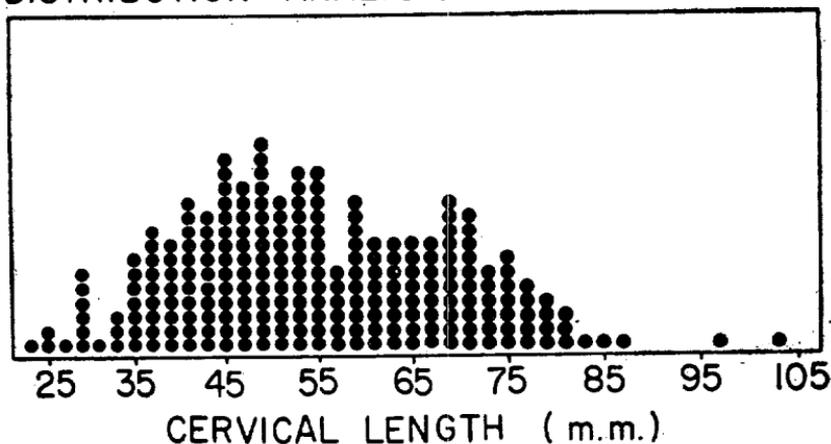


Fig. IX.

In conclusion I wish to point out that the main object of this investigation up to now has been to demonstrate the existence of the problem, to attempt to define it within certain limits and at the same time endeavour to develop some of the techniques and procedures which may subsequently prove useful in the ultimate solution of the problem.

This investigation is continuing but many aspects have yet to be defined before we can consider trying to discover the various causes and their elimination; but it is felt that even at this stage the greatest potential for improvements of fertility in our sheep lies in a more careful study of the contribution of the female rather than the male in this joint enterprise.

**Acknowledgments.**—Sincere thanks are accorded to the Nuffield Foundation whose financial assistance permitted a sufficiently large number of animals to be examined during the 1955 breeding season for worth-while conclusions to be drawn from the results obtained. Thanks are due also to Miss N. Laffey who has rendered valuable assistance in handling much of the routine work entailed.

### References:

- Blandau, R. J. (1952) *Fertility and Sterility* 3, 349.  
Brock, H. and Rowson, L. E. (1952) *J.Agric.Sci.* 42, 479.  
Dutt, R. H. (1954) *J.Anim.Sci.* 13, 464.

# Discussion

Dr. SKALLER: What variation occurs in the production of normal and abnormal eggs between different animals?

Mr. HART: To date we have not been in the position to analyse ova production on a breed or species basis. All the data presented refers to grade Corriedale and purebred Corriedale ewes, therefore it is hardly possible to answer this question at this stage.

Dr. EDGAR: I would like to congratulate Mr. Hart on tackling a problem bristling with difficulties as well as with promise and in addition on the amount of work which he has done so far. However, one very obvious difficulty is to determine whether abnormalities which are seen under the microscope are real or artefacts produced by the handling of the material. Again, where ova appear to be undergoing involution, can we be sure that this is because they are not fertilisable or simply because they have not been fertilised?

Mr. HART: It is our opinion that involution prevents fertilisation taking place and is not the result of the ova not becoming fertilised. This can be substantiated to some extent by the fact that involuted ova have been found in all positions in the oviduct. If the present concept that normal ova are still fertilisable in, say, the lower third of the oviduct is accepted then involuted ova recovered from the upper third of the tube have obviously undergone involution long before they had reached the limits of fertilisability, and so could not have, as Dr. Edgar suggests, then become involuted as the result of not having been fertilised.

Dr. EDGAR: Supposing the egg has been shed from the ovary too long before insemination took place, would you not expect to find the involution of the egg had taken place just because fertilisation had not occurred?

Mr. HART: No, all our evidence points to the exact opposite, namely that fertilisation fails to take place because of involution.

Dr. EDGAR: When fertilisation has taken place and the egg subsequently degenerates, the ram is not necessarily exonerated because he contributes half of the nuclear material and this may have come from an abnormal sperm.

Mr. HART: This may well be true if we are sure that an abnormal sperm is capable of penetrating and fertilising an ovum.

Dr. WALLACE: Would Mr. Hart elaborate on what he means by disintegration of an unfertilised egg? At Ruakura a few years ago, I ran teaser rams with a mob of ewes when there was no chance of eggs being fertilised. I was able to recover eggs up to twelve days after mating without any evidence of the cell membrane disintegrating. If, as Mr. Hart suggests, 17 per cent of the eggs shed are abnormal, it is surprising that on a flock basis over 80 per cent of ewes conceive at the first heat.

Mr. HART: In our experience, the zona pellucida of an unfertilised egg may remain whole for considerable lengths of time. The nuclear membrane weakens first, often quite early, and allows some of its contents to permeate through into the cytoplasmic space without any apparent visual damage to its actual structure. Later as disintegration proceeds the nuclear membrane disappears entirely and there is just one mass of formless disintegrating material enclosed within the zona pellucida. Regarding the second part of the question, the 17 per cent abnormal eggs is the mean abnormal egg

production, over the whole of the breeding season, which was in this case over four months. An analysis of the data, which time did not permit in presenting this paper, shows that during the first six weeks of the breeding season, the period covering the 80 per cent conception at first heat statement, the percentage abnormal to be at its lowest, namely 11.5 per cent. This, I feel, in no way conflicts with the flock conception rates at first heat, particularly where multiple ovulations have also to be considered.

Mr. McFARLANE: Before making such strong claims for the part the female is playing in infertility and before accepting the fact that his abnormalities are in fact abnormalities, Mr. Hart should have shown us some systematics of the process of involution and death. I am perturbed that the microscopic techniques used were not adequate to accurately show the structures photographed. I would rather see the ova classified according to their structure and the position in which they were found.

Mr. HART: In replying to this question, I should point out that we fully appreciate there may be weakness in our present techniques. This is admitted, also that this work as pointed out in the text is in the preliminary stages of development. Nevertheless, with the experience of six years, and the handling of more than a thousand ova, we feel that there is no doubt that these abnormalities are in fact abnormalities. Classification according to ovum structure will have to await more facilities. The position where found has been recorded and will be incorporated in future analyses.