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THE SIGNIFICANCE OF THE CYTOPLASMIC DROPLET IN THE DISINTEGRATION OF SEMEN IN GUERNSEY BULLS

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INTRODUCTION

During the examination of semen from a total of 463 bulls including Guernseys, Hancock & Rollinson (1949) encountered an abnormality of the semen of 12 Guernsey bulls. This was characterized by complete separation of the head of the sperm from the tail in virtually all the spermatozoa. The affected bulls were completely sterile. Examination of the pedigrees of the 12 bulls failed to show any close relationship, the only ancestor common to all being seven generations removed in one instance.

Hancock (1955) subsequently showed that disintegration in these cases occurs during the passage of the spermatozoa through the caput epididymis and is associated with the migration of the residual cytoplasm from the neck of the spermatozoon to the distal end of the middle-piece. He further showed that the migration of the cytoplasmic droplet occurs almost exclusively in the caput epididymis.

Alun-Jones (1962) reports seven cases in the Island of Guernsey, and states that all seven were descendants of one sire. As a result of these findings the Royal Guernsey Agricultural and Horticultural Society ruled that as from 1April 1960: "All bulls aged 12 months or over at the date of sale, which have not yet proved themselves, must be examined for fertility by a Veterinary Surgeon before being sold."

Guernsey cattle have been exported to many parts of the world, yet the defect has thus far been reported only in Britain and the Island of Guernsey. The present investigation was commenced in 1959 after reports of infertility in bulls from some Guernsey studs. Though it was found to be basically the same condition as that described overseas, important differences were revealed and additional information was obtained on the pathogenesis of the defect.

In associating sperm disintegration with migration of the cytoplasmic droplet, Hancock (1955) states that the significance of this association is not clear. He postulated that the migrating droplet may fail in separating itself from the head and actually tear the head from the middle-piece or that the droplet merely holds the head in position for a time after the normal connections of the head and tail have been severed. He concludes that his postulates do not explain the real nature of the defect.

The possibility of the cytoplasmic droplet causing infertility prompted a study of its behaviour in normal bulls and rams in order to ascertain in which ways its migration may cause sperm disintegration.

The frequent observation of a similar type of disintegration of the sperm in rams after a febrile reaction to bluetongue vaccine provided an additional incentive for this study.

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Materials and Methods

Semen from five affected bulls (No. 1 to 5) in one particular stud (Stud A) was obtained for examination. Bull No. 3 from this stud was acquired and studied at this Institute over a period of three years. The observations embraced a study of his sexual behaviour, repeated examination of sperm morphology, determination of survival rate, metabolic activity and citric acid and fructose content of sperm, and exhaustion tests. When he was eventually slaughtered, stained smears from the rete testis as well as from the seven sites indicated in Fig. 1 were examined microscopically.

In addition to the five bulls from Stud A, eight others of the same breed and from various parts of the country were brought to the authors' notice either through semen samples or smears sent in for examination or by reports from veterinarians who had examined the animals and their semen.

Studies on the cytoplasmic droplet were made on five normal bulls and two rams.

In a live bull 0·2 ml semen was drawn off under aseptic conditions from the caput epididymis at two different sites with a gauge 17 sterile needle, and examined immediately under the phase contrast microscope.

The testes of the second bull were removed immediately after the animal had been killed with a humane killer, and semen from different sections of the epididymis was examined instantly under the phase contrast microscope.

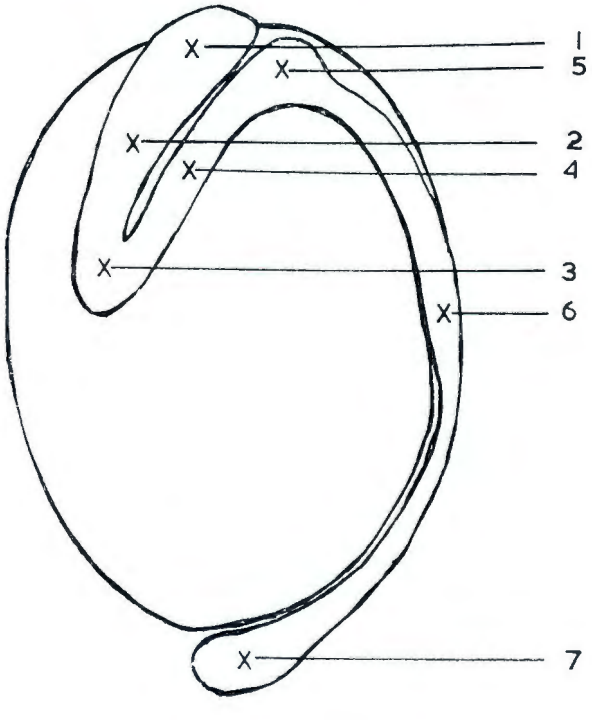

Fig. 1.—The seven sites on the epididymis from which semen samples were obtained

The testes of the other three bulls and two Merino rams were also recovered immediatley after slaughter, and sections from the epididymides taken from the seven sites indicated in Fig. 1. These were squashed in 3 ml saline at body temperature and a drop from each section was examined on the spot under phase contrast microscope. At the same time smears were made and stained with eosin-nigrosin for subsequent examination.

Determination of the fructose content and calculation of the " fructolysis index " were done according to Mann (1948).

Results

The results of microscopic examination of the semen of the five bulls in Stud A and of semen smears submitted from five other sires, are given in Table 1.

TABLE 1.—*Differential count of spermatozoa of ten Guernsey bulls*

Bull number	Normal sperm	Disintegrated sperm	Other head abnormalties	Tailless sperm	Coiled tails
	%	%	%	%	%
1.....	40	47	3	0	10
2.....	22	55	12	6	5
3.....	12	72	12	—	4
4.....	8	78	10	2	2
5.....	12	68	6	10	4
9.....	65	12	18	0	5
10.....	22	4	38	0	36
11.....	32	55	10	0	3
12.....	0	100	0	0	0
13.....	1	99	0	0	0

In this table " other head abnormalities " comprise all aberrations in the sperm head resulting from faulty spermatogenesis, such as pyriform heads, narrow heads, round heads, microsperm, megalosperm and abaxial attachment of the tail to the head. Although the latter defect on its own is considered by many not to cause serious impairment of fertility, the frequency with which it occurs in these bulls raises the question as to whether it may not predispose to separation between head and tail in those animals with this inherent tendency.

" Tailless sperm " in Table 1 designates spermatozoa with the neck intact and the middle-piece attached to the head but the tail missing. This unusual abnormality was observed in three of the five bulls in Stud A.

The percentage disintegrated sperm (loose heads) varied from 4 to 100 but in most instances it was far below the proportion reported by Hancock & Rollinson (1949).

Not much definite information could be obtained on the fertility of some of these sires, but it is certain that they were not all completely sterile. No. 1 was the herd sire in Stud A, and had produced several offspring including No. 2, 3, 4, 5, 8, 10 and 13. His fertility at that time was on the wane, and eight months later he was disposed of for slaughter.

The purchasers of No. 2 and 8 returned them to the breeder with the complaint that, although having sired a few calves, they were very poor breeders.

No. 9 was disposed of because the breeding results in the herd served by him were not good. His subsequent breeding record is not available.

The owner of No. 11 complained that, after he showed normal fertility in previous years, the cows served by this six year old sire were returning four and five times, and even then some did not conceive. He was consigned to the abattoir after the owner had received the report on the semen examination.

A veterinarian submitted semen smears and a report on No. 12. An examination of the smears showed 100 per cent disintegrated sperm and his breeding record revealed that he was completely sterile at the time although the owner declared that he had previously sired two calves.

The remaining seven animals (No. 3, 4, 5, 6, 7, 10 and 13) were all apparently completely sterile and most of them had been returned to their respective breeders on this account.

Observations made on bull No. 3 at this Institute over a three year period established that his sexual behaviour was completely normal in every respect. Actually his libido was well above that of the average normal sire, and no difficulty was experienced in collecting semen from him with an artificial vagina at any time. Notwithstanding the fact that collections were made twice or three times a week throughout the year, he served readily when the two exhaustion tests were made.

Disintegration of the sperm rapidly became worse after his arrival at this Institute and was practically hundred per cent within four months after the semen reported on in Table 1 was obtained.

Repeated examinations of freshly collected semen made under a phase contrast microscope revealed that the detached heads were motionless while many of the loose tails showed considerable activity in the course of which a cytoplasmic droplet or a loose head was frequently caught up in a loop of the tail. This gave a very close resemblance to a normal intact spermatozoon for which it may be mistaken on cursory examination (Fig. 2).

In the first exhaustion test he yielded 22 ejaculates in 85 minutes, these varying in volume from 1·7 ml to 5·6 ml. A total volume of 74·7 ml was obtained from him. The density of the 22 samples was rated as follows: 3 milky, 13 thin milky and 6 watery. Smears were made from each sample and stained with nigrosin-eosin. Numerous sperm were present in every sample. Counts of 200 sperm heads were made in each smear, and of the 4,400 thus observed only four intact sperm were seen. One of these was in the tenth, two in the eleventh and one in the twelfth ejaculate. One sperm in the 22nd ejaculate had the middle-piece attached but no tail.

The brief interval between successive ejaculates did not permit of a more detailed examination of each. For that reason a more comprehensive study was made of only nine samples in the second exhaustion test which was carried out nine months after the first. In this test 25 ejaculates were obtained in 262 minutes, the volume varying from 1·2 ml to 7·0 ml. The total was 67·1 ml. Density was fairly constant throughout.

The results of the detailed examination are given in Table 2.

The fructose content was not below normal limits in any one of the nine samples. On the contrary it was rather above average in many samples which is evidence of high luteinizing hormone activity and probably accounts for the unusually high libido displayed. The pH rose progressively from 6·8 to 8·1.

As in the first test, only four intact sperm were seen. The abnormal heads consisted predominantly of tapered heads although narrow and pyriform heads were also frequent.

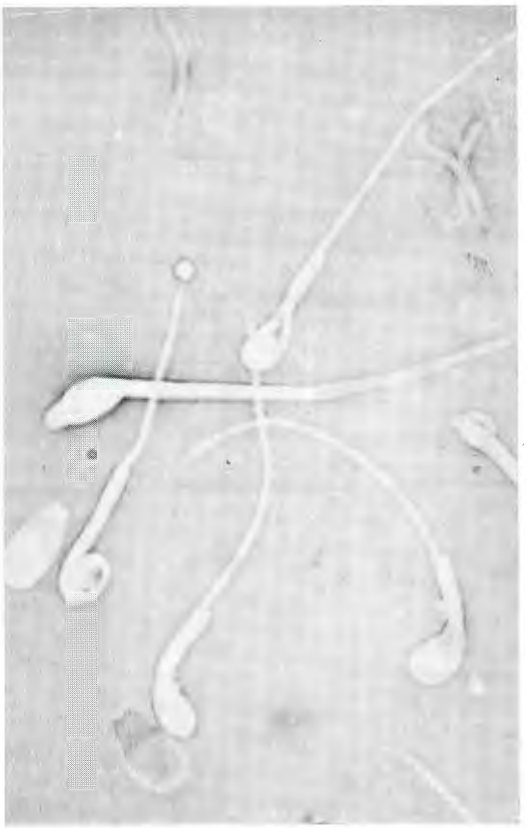

FIG. 2.—Disintegrated sperm of bull No. 3

TABLE 2.—*Second exhaustion test of Guernsey bull number* 3

Ejaculate number	Volume	Fructose	pH	Motile tails	Intact sperm	Abnormal heads	Abnormal tails	Live tails	Live head
	ml	mg/100 ml		%	%	%	%	%	%
1.....	5·0	866	6·8	25	0	8	76	39	35
5.....	4·0	731	6·9	60	1	4	67	71	46
7.....	4·0	646	7·0	70	0	4	62	64	50
10.....	2·5	478	7·2	25	1	5	53	28	14
13.....	1·3	373	7·6	0	1	6	28	4	2
16.....	2·0	277	7·8	0	1	10	27	0	0
19.....	2·5	158	7·9	0	0	6	22	0	0
22.....	2·0	276	7·5	20	0	5	32	21	14
25.....	1·2	311	8·1	0	0	4	34	0	0

Smears stained with Giemsa showed the presence of considerable amounts of irregular cytoplasmic masses in all ejaculates. Small pyknotic chromatin-like masses were present in all ejaculates after the tenth. Initially a small band of pyknotic basophylic material was seen on the head at the neck junction. In the last few ejaculates this was evident as several granules independent of each other.

Some distal droplets were present in the first few ejaculates but were absent thereafter.

Tail abnormalities consisted largely of bent and loosely coiled tails.

Three successive ejaculations were taken for the determination of metabolic activity and the results of the observations are detailed in Table 3.

TABLE 3.—*Metabolic activity of spermatozoa of bull number* 3

	Ejaculate number		
	1	2	3
Volume	7·4	7·0	5·4
Heads per ml × 10^6	1425	1113	706
Tails per ml × 10^6	1387	1100	713
Mass Motility (0–5)	1+	−2	2
pH—0 hr	6·8	6·85	6·85
0·5 hr	6·6	6·65	6·8
1·0 hr	6·5	6·5	6·65
1·5 hr	6·4	6·4	6·55
2·0 hr	6·3	6·25	—
Fructose—0 hr	697	347	362
1 hr	618	253	362
2 hr	507	208	256
Total fructose (mg per ejaculate)	51·6	24·3	19·6
Fructose index: 0–1 hr	0·56	0·85	1·41
1–2 hr	0·79	0·41	0·06
Citric acid concentration	721	868	897
Total citric acid	53·4	60·8	48·4

Two other ejaculates taken subsequently were pooled and centrifuged slowly for five minutes. Samples were taken from the supernatant portion (A) and from the sediment (B) for fructolysis determination and counts of the relative concentration of sperm heads and tails. The results are given in Table 4.

TABLE 4.—*Observations on centrifuged sample of spermatozoa of bull number* 3

	A—Supernatant fluid	B Sediment
Concentration of heads (× 10^6)	0,043 (16%)	0,720 (62%)
Concentration of tails (× 10^6)	0,230 (84%)	0,445 (38%)
Fructolysis index: 0–1 hr	1·68	0·017

The biggest concentration of heads was in the sediment while tails predominated in the supernatant portion. Associated with this distribution of the two component parts of the spermatozoon is the fact that fructolysis as determined by the index was normal in Sample A which contained 84 per cent tails and was almost negligible in B. Such fructolysis as did occur in B can be attributed to the small percentage tails it contained. This observation indicates that fructolysis in semen is mainly confined to the tail which is the organ responsible for all motility shown by the sperm and that the role played by the head in fructolysis is almost negligible.

The results yielded by the various tests carried out on bull No. 3 indicate that, with sperm concentration and exhaustion tests as criteria, the rate of spermatogenesis is normal. The pH and the citric acid and fructose levels indicate normality in the seminal plasma. This is evidence of normal pituitary gonadotrophic activity and androgenic function.

The degree of fructolysis and the pH shift show that the rate of metabolism of the decapitated spermatozoa is only slightly below that of normal spermatozoa.

The survival rate of the separate heads and tails in the male genital tract is equal, indicating that there is no selective absorption or phagocytosis of either head or tail.

When this bull was eventually killed, the testes and epididymides were removed immediately and treated as described by Hancock (1955), with the exception that samples of spermatozoa were also taken from the rete testis, the body and the tail of the epididymis, and from the five sites from which collections were made by Hancock. Smears made from each of these eight sections were stained with methyl violet and differential counts of 200 to 300 spermatozoa were made in each smear. The results are given in Table 5.

The most significant feature brought to light is that in this particular case decapitation occurred mainly in the rete testis where 51·5 per cent sperm were disintegrated and only 29·5 per cent were intact, the remaining 19 per cent having broken necks. Hancock (1955), on the other hand, found in his investigation that the majority sperm were still intact when they reached site 1 in the caput epididymis.

Disintegration continued rapidly in sites 1 to 3 and no intact sperm were found after they had passed through the curvature of the caput at site 3.

All the intact sperm in the rete testis and at site 1 had cytoplasmic droplets, and it is noteworthy that in every case the droplet had not moved further down than the neck of the sperm.

In the disintegrated group, where only the tails were counted, the number of tails with no cytoplasmic droplets increased progressively from the rete to site 7 and it can be presumed that in these the neck droplets were discarded at the time separation between the head and tail occurred.

The role played by the cytoplasmic droplet

Both Hancock's (1955) findings and the results of the present investigation strongly suggest that the cytoplasmic droplet plays a major role in sperm disintegration. A consideration of the origin of this seemingly superfluous mass of protoplasmic material and its association with the sperm is, therefore, necessary for proper understanding of the mechanism involved in sperm disintegration.

TABLE 5.—*Differential counts of spermatozoa from the rete testis and various parts of the epididymis of bull number* 3

Site	Intact sperm	Disintegrated sperm	Broken necks	Intact sperm with		Disintegrated sperm with			
				No droplets	Droplets on neck	No droplets	Droplets on proximal middlepiece	Droplets on distal middlepiece	Droplets on tail
	%	%	%						
Rete testis	29·5	51·5	19	0	29·5 (100%)	5·5 (10·7%)	45 (87·4%)	1 (1·9%)	0
1	26	54	20	0	26 (100%)	12 (22·2%)	42 (77·8%)	0	0
2	8·5	77·5	14	0·5 (5·9%)	8 (94·1%)	26·5 (34·2%)	50·5 (65·2%)	0·5 (0·6%)	0
3	1·3	93	5·7	0·4 (30·8%)	0·9 (69·2%)	31·7 (34·1%)	59 (63·4%)	2·3 (2·5%)	0
4	0	99·3	0·7	0	0	42·4 (42·7%)	32 (32·2%)	23·7 (23·8%)	1·2 (1·3%)
5	0	99·6	0·4	0	0	51 (51·2%)	5 (5·0%)	43 (43·2%)	0·6 (0·6%)
6	0	99·6	0·4	0	0	54·5 (54·7%)	1·8 (1·8%)	42·6 (42·8%)	0·7 (0·7%)
7	0	99·5	0·5	0	0	55·9 (56·2%)	2·6 (2·6%)	41 (41·2%)	0

During spermiogenesis, which is the second phase of spermatogenesis, the spermatids undergo metamorphosis resulting in the completely formed, but still immature, spermatozoa. The changes include the formation of the acrosome, head, middle-piece and tail and of their constituent parts from the various cellular materials. During this process some of the cytoplasm of the spermatid is excluded from the final sperm cell and becomes the cytoplasmic droplet, which is regarded as a characteristic of immature sperm.

Salisbury & Vandemark (1961) state that the granular Golgi bodies remain at the neck of the fully formed spermatozoon and after elimination of the excess cytoplasm a residual protoplasmic droplet or bead containing argentophilic granules is left. The bead is not usually lost until the spermatozoon has completed at least part of its journey through the epididymis.

Examination of fresh live sperm under the phase contrast microscope immediately after collection from two sites in the caput epididymis of the normal, live bulls as well as from various sections of the epididymis of the first normal bull to be slaughtered, revealed remarkable activity and lashing or oscillating movements of the tails of all those spermatozoa that still had the cytoplasmic droplet, while those that were free from it were lying still. A significant observation was that, as soon as the bead had been shaken off, these vigorous tail movements ceased and the sperm became motionless. Differential staining with nigrosin-eosin showed that they did not die after discarding the droplet. The whole picture is that of a determined effort made by the sperm in an attempt to shake off the droplet which must be regarded as a foreign body at that stage. An important feature of the type of motility exhibited by sperm in the epididymis is the complete absence of progressive linear movement, the activity consisting merely of oscillating or lashing movements of the tail.

By observing a drop of live sperm from the caput on a warm stage under the phase contrast microscope for a few minutes, the whole process of unloading of the cytoplasmic droplet could be clearly seen. It was noticed too that in the majority the droplets did not pass along the entire length of the tail, but were discarded at the distal end of the middle-piece and the proximal extremity of the tail. This observation is confirmed by the complete absence or the relatively small percentage of droplets on the tails of the sperm throughout the entire length of the epididymis as recorded in Table 6.

The data pertaining to the three normal bulls and ram No. 1 in Table 6 reveal no significant difference in the percentage of sperm with cytoplasmic droplets in sites 1 to 3, but with one exception there is a marked decrease in this type of sperm in the subsequent sites. The indications are, therefore, that most of the sperm divest themselves of the cytoplasmic droplet in the curvature of the caput (site 3).

The position of the cytoplasmic droplet on the sperm in the different sections of the epididymis is indicated by the seven photos in Fig. 3.

The figures for ram No. 2 are interesting in that they suggest an apparent inability of the sperm to rid themselves of the droplet, and, moreover, that the movement of the droplet from the neck to the tail was abnormally slow. It can only be surmised that there was some unknown factor which inhibited or retarded motility of sperm in this animal.

Attention must also be drawn to the fact that ram No. 2 was the only one of the five sires in which 100 per cent of the sperm still had cytoplasmic droplets when they reached the caput. It would thus appear that some unloading of the droplets had already taken place in the rete testis of the other four.

TABLE 6.—*Distribution of cytoplasmic droplets on sperm in various sections of the epididymides of three normal bulls and two normal rams*

Animal	Site	Percentage of sperm in which droplets are					
		(*a*) Absent	(*b*) Present	Number of droplets located on			
				neck	proximal middle piece	distal middle piece	tail
		%	%	%	%	%	%
Bull 3.....	1	10	90	80	4	3	3
Bull 4.....		5	95	92	3	0	0
Bull 5.....		8	92	87	3	2	0
Ram 1.....		52	48	43	1	4	0
Ram 2.....		0	100	100	0	0	0
Bull 3.....	2	4	96	94	0	2	0
Bull 4.....		1	99	97	2	0	0
Bull 5.....		36	64	20	9	30	5
Ram 1.....		60	40	36	0	4	0
Ram 2.....		2	98	96	2	0	0
Bull 3.....	3	7	93	76	5	11	1
Bull 4.....		10	90	40	8	42	—
Bull 5.....		18	82	71	7	3	1
Ram 1.....		31	69	52	10	7	0
Ram 2.....		1	99	94	5	0	0
Bull 3.....	4	54	46	16	8	13	9
Bull 4.....		9	91	16	7	64	4
Bull 5.....		41	59	8	8	41	2
Ram 1.....		80	20	6	—	14	0
Ram 2.....		2	98	56	22	20	0
Bull 3.....	5	46	54	11	7	32	4
Bull 4.....		53	47	11	4	30	2
Bull 5.....		49	51	5	5	28	13
Ram 1.....		82	18	2	2	14	0
Ram 2.....		34	66	0	4	62	0
Bull 4.....	6	83	17	1	3	10	3
Bull 5.....		71	29	5	2	17	5
Ram 1.....		60	40	0	0	40	0
Ram 2.....		34	66	0	0	66	0
Bull 4.....	7	81	19	4	1	12	2
Bull 5.....		67	33	2	1	26	4
Ram 1.....		99	1	0	0	0	1
Ram 2.....		14	86	0	0	86	0

These conclusions are based on the presumption that spermatozoa in the rete testis have the capacity to show motility and it raises the controversial question as to the exact site at which this characteristic is acquired, since there is a fairly widespread belief that sperm in the epididymis are immotile. On the other hand, Young (1929), after a detailed investigation, concluded that the capacity for movement is acquired in the testis and Bishop (1961) stated that testicular spermatozoa of the bull are frequently motile.

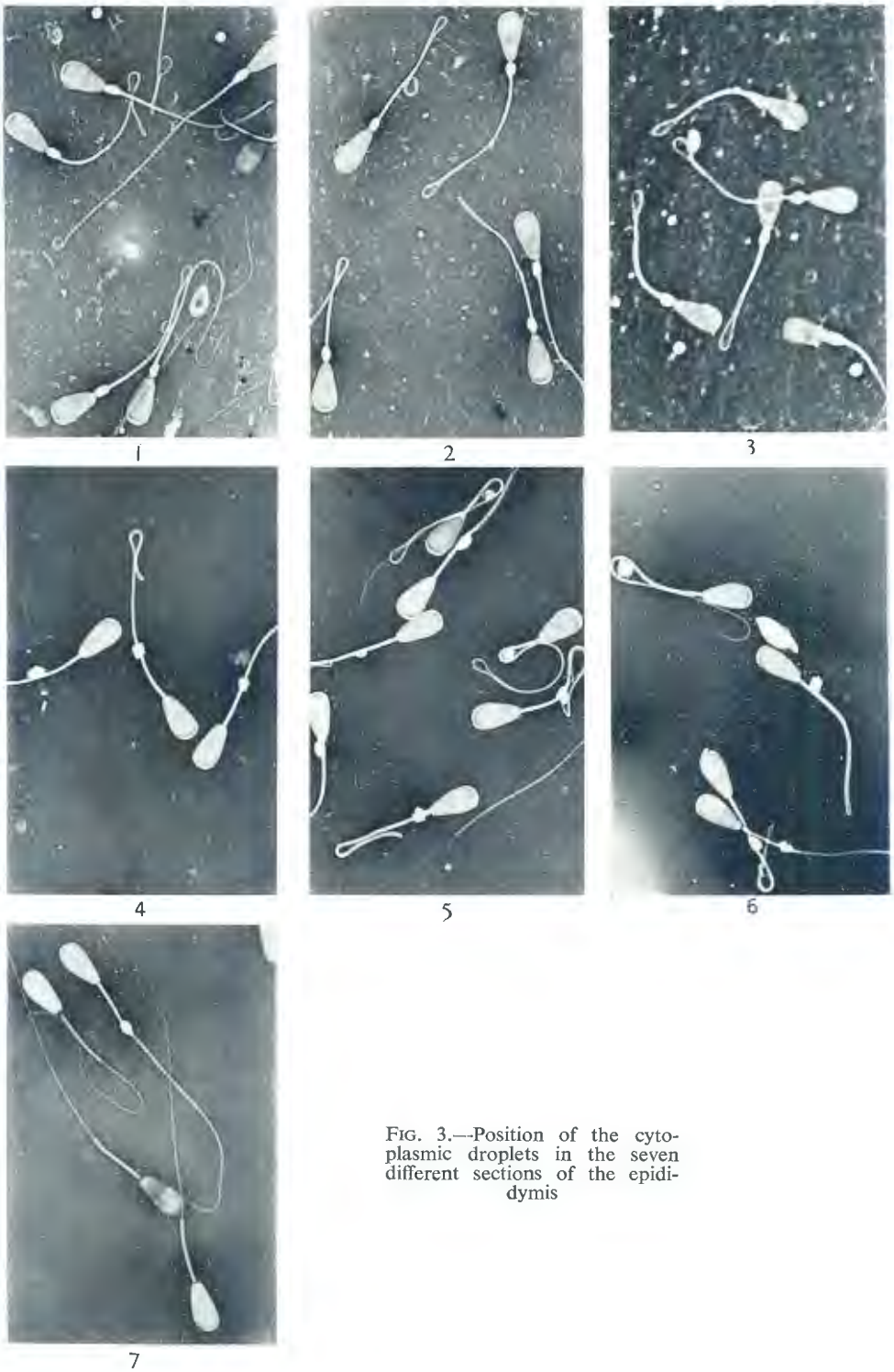

FIG. 3.—Position of the cytoplasmic droplets in the seven different sections of the epididymis

Misconception is apparently produced by confusing the ability to display movement with the actual act of motility. The statement that sperm in the epididymis are immobile does not necessarily imply that they are not capable of exhibiting motility. The question of motility exhibited by epididymal spermatozoa is also sometimes confounded by not differentiating between linear and other types of motility.

It has been demonstrated in this work that epididymal sperm rid themselves of the cytoplasmic droplet by vigorous oscillating movements of the tail and, when this has been accomplished, they lapse into a quiescent state. It is thus concluded that while sperm in the rete testis and epididymis are capable of displaying motility, they are normally in a state of rest and only display activity when provoked thereto by unfavourable conditions such as the presence of a foreign body like the cytoplasmic droplet.

Discussion

The results obtained in this investigation indicate that the condition occurring in certain bulls in South Africa is fundamentally the same as that studied by workers in Great Britain. There are nevertheless important differences, and consideration of these may assist in elucidating the aetiology and pathogenesis of sperm disintegration.

With few exceptions the percentage disintegrated sperm is not as high as noted in the British animals, only two of the ten in Table 1 showing over 80 per cent disintegration. A very significant fact emanates from this, viz. that six of the thirteen bulls investigated showed some degree of fertility in the early stages of their careers whereas all the affected sires in Britain were completely sterile from the start. The data presented and the available information on the breeding performance suggest that the condition is progressive and that in these cases with a comparatively low proportion of affected sperm, the tendency to disintegrate increases rapidly. For instance bull No. 3 showed only 78 per cent sperm with loose heads when he was first examined, but within four months it was practically 100 per cent, and frequent examination of his semen over the next three years rarely revealed an intact spermatozoon.

This seminal deterioration was naturally accompanied by rapid lowering of reproductive capacity in those affected bulls that had displayed some degree of fertility initially

Another point on which the results are not in complete agreement with those obtained in Britain is the site at which disintegration occurs. Hancock (1955) found that most of the sperm disintegrate during their passage through the caput epididymis whereas the data in Table 5 show that in the one bull that was available for post mortem study in this investigation, 51·5 per cent of the spermatozoa in the rete testis were already decapitated.

The site where most sperm heads become detached probably varies from animal to animal and may be determined to a large extent by the degree of weakness of the neck of the sperm. It is conceivable that in this case the neck was particularly frail, resulting in decapitation in the majority of spermatozoa as soon as motility was initiated in the rete testis. In this connection the relatively large percentage of broken necks (19) found in the rete testis is significant.

As regards the actual mechanism fo decapitation, the observations on both affected and normal animals support Hancock's contention that separation between the sperm head and tail is associated with the presence of the cytoplasmic droplet. The additional studies on the droplet in normal bulls and rams, however, suggest that it results, not from the droplet tearing the head away from the middle-piece and tail,

but rather from the tail pulling itself and the middle-piece away from the head during the violent struggling of the sperm in its endeavour to divest itself of the cytoplasmic droplet.

According to unpublished data on studies made at this Institute on the semen of rams that have reacted to vaccination against bluetongue, disintegration of sperm is a prominent feature and it is not uncommon to find over 90 per cent loose heads. This is generally accompanied by primary abnormalities indicating interference with spermatogenesis, and it may take six to eight months before normality is restored.

The opinion that disintegration is produced by excessive movement on the part of the sperm in an attempt to discard the cytoplasmic droplet, is based on the presumption that the connexion between head and tail is abnormally delicate in such cases. Pronounced activity is shown by all normal spermatozoa soon after reaching morphological maturity and while the cytoplasmic droplet is still attached.

It is probable that in some infectious febrile diseases where the testes are also invaded by the viral pathogens, spermatogenesis is disturbed to such an extent that various defects in sperm morphology are produced. One of these is the weak neck which is liable to be broken off when the foreign body effect of the droplet on the neck stimulates sperm activity. This does not, however, explain the disintegration of otherwise completely normal sperm in healthy Guernsey bulls in which all the tests point to normal spermatogenesis, and it can only be concluded that in these cases the spermatozoa have a congenital defect of the attachment between head and tail. This gives rise to separation between these two components as soon as motility commences.

The specificity of this abnormality for the one particular breed immediately raises suspicion of a genetic origin. This investigation has produced strong evidence in support of the view of overseas workers that the defect is probably hereditary, since the available pedigrees reveal a close relationship between the affected bulls.

Seven of the animals investigated were sired by bull No. 1 which showed 47 per cent disintegrated sperm, and was disposed of on account of poor fertility. Three blood lines figure very prominently in the pedigrees of the parents of all affected animals, and according to D. H. L. Rollinson in a personal communication (1959) two of the three also frequently occur in the pedigrees of the cases studied in Britain.

The available information indicates that the defect is due to a recessive factor carried by the sires and the dams of affected bulls. The heterozygous nature of this abnormality associated with the fact that some bulls possess a degree of fertility in the early stages, may render its elimination difficult, but not impossible, provided careful selective breeding is carried out and the ruling of the Royal Guernsey Agricultural & Horticultural Society is strictly adhered to.

Summary

The occurrence of sperm disintegration in Guernsey bulls in South Africa was investigated in thirteen animals that were either sterile or displayed low fertility. One of these was kept under observation and subjected to repeated tests and examinations for three years.

In an attempt to elucidate the mechanism whereby the sperm head was detached from the tail, the sperm activity in the epididymis, and particularly the discarding of the cytoplasmic droplet, were studied in three normal bulls and two normal rams.

In ten affected bulls the percentage disintegrated sperm varied from 4 to 100. In several this percentage was not as high as that reported in Britain and the animals showed some degree of fertility in the early stages, but the condition deteriorated with ageing.

Two exhaustion tests were carried out on one bull. These yielded 22 and 25 ejaculates in 85 and 262 minutes totalling 74·7 and 61·7 ml semen respectively. In each test only four intact sperm were found.

The tests further showed that spermatogenesis was normal as regards the number of sperm formed, while pH and the citric acid and fructose levels were also normal in the seminal plasma.

A determination of fructolysis activity and pH shift showed that the metabolic rate of disintegrated sperm was similar, or only slightly below that of normal sperm. Their survival rate indicated that there was no selective absorption or phagocytosis of either heads or tails in the male genital tract.

On centrifugation of semen the biggest concentration of heads was in the sediment and tails predominated in the supernatant fluid. The tails of the sperm are mainly responsible for fructolysis.

In the bull available for post mortem study, an examination of sperm from different sites showed that disintegration occurred mainly in the rete testis and curvature of the caput epididymis.

Examination of live sperm from different parts of the caput epididymis immediately after collection disclosed considerable activity characterized by violent lashing of the tails in all the spermatozoa with a cytoplasmic droplet. This activity ceased as soon as the droplet was discarded. Most of the droplets are unloaded from the distal end of the middle-piece and few reach the tail. The discarding of the droplets occurs mainly in the curvature of the caput epididymis.

It is concluded that in those animals with an abnormally weak attachment between the sperm head and tail, caused by congenital defect or a pathological condition, separation between the two components occurs in the rete testis and caput epididymis during the severe lashing movements made by the sperm in its efforts to cast off the cytoplasmic droplet.

The evidence indicates that in affected Guernsey bulls the defect is attributable to a recessive hereditary factor.

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