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THE percent of abnormal sperm in a semen sample has been considered as one index of fertility. A more accurate interpretation of abnormal types would improve the value of this index. Mercier and Salisbury (1947) demonstrated that the proportion of tailless sperm in smears of bulls' semen was increased by clearing the slides in chlorazene, or decreased by storing the semen for 4 days before making smears. Emik and Sidwell (1947) found the opal blue method to be superior to techniques of smearing followed by staining, principally because it produced fewer abnormal sperm, permitted an estimation of live sperm, and made a stained slide immediately available for examination.

Data compiled during the latter investigation were adaptable to tracing some of the relationships between proportions of tailless sperm and techniques. These relationships were combined with other observations to obtain a means of estimating the proportions of tailless sperm produced in vivo or created in vitro.

Materials and Methods

Most of the data for this report were collected at the same time and from the same rams under the conditions described by Emik and Sidwell (1947). Counts of tailless sperm were made by two workers from three sources: (1) with a hemacytometer from 1:200 dilutions of semen in phosphate buffered solutions of opal blue and eosin, with minute quantities of chlorazene, formalin, or phenol added to some preparations to inhibit motility; (2) from 1:200 dilutions of the same ejaculates in 4 percent chlorazene; and (3) for each sample from opal blue stained smears.

Percentages of tailless sperm were determined from counts of 100 sperm. These percentages, for each individual count, were transformed for use in analysis according to the method proposed by Bartlett (1947) for normalizing their distribution. The table originated by C. I. Bliss and published in Snedecor (1946) was used to transform percentages to angles in degrees.

The transformed means of the data from dilutions with phosphate buffered solutions of opal blue and eosin, or dilutions with chlorazene, ($\bar{X}$'s), were compared with the transformed means from the opal blue smears, ($\bar{Y}$), by the complete t formula:

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$$t = \frac{\bar{x} - \bar{Y}}{\sqrt{\sigma_x^2 + \sigma_y^2 - 2r\sigma_x\sigma_y}}.$$

Analyses of covariance were also made following the methods given in Snedecor (1946).

Results and Discussion

The comparisons of means of the transformed data are given in table 1. The percentages of tailless sperm from the opal blue smears were signifi-

TABLE 1. PERCENT TAILLESS SPERM IN PHOSPHATE BUFFERED OR CHLORAZENE DILUTIONS IN THE HEMACYTOMETER (X) COMPARED WITH OPAL BLUE STAINED SMEARS (Y), PERCENT TRANSFORMED TO ANGLES

Semen quality	Preparation	Ram no.	Transformed varieties						Actual percents	
			n	$\bar{X}$	$\bar{Y}$	σ_D	t	r	$\bar{x}$	$\bar{y}$
Good	Phosphate	142RW	15	8.59	8.51	2.14	1	−.20	2.23	2.19
		2260W	8	9.90	8.49	0.96	1.47	.64	2.95	2.18
		Sum	23	9.05	8.50	1.33	1	.07	2.48	2.18
	Chlorazene	142RW	13	9.22	9.72	1.24	1	.08	2.57	2.85
		2260W	7	6.90	9.19	1.47	1.57	.68	1.45	2.55
		Sum	20	8.41	9.54	0.93	1.21	.38	2.14	2.75
Total good			43	8.75	8.98	0.82	1	.19	2.32	2.44
Poor	Phosphate	88RW	12	45.93	51.39	2.26	2.42*	.90**	51.6	61.1
		384RW	15	46.09	53.22	1.29	5.53**	.68**	51.9	64.1
		Sum	27	46.02	52.41	1.25	5.10**	.87**	51.8	62.8
	Chlorazene	88RW	11	46.94	51.35	2.32	2.32*	88**	53.4	61.0
		384RW	12	49.08	54.04	1.19	4.18**	.75**	57.1	65.5
		Sum	23	48.05	52.75	1.25	3.75**	.86**	55.3	63.4
Total poor			50	46.96	52.57	0.86	6.48**	.86**	53.4	63.1

* probability less than .05.
** probability less than .01.

cantly higher than those from either dilution, for the rams with semen of poor quality. There were no significant differences between means for the rams with semen of good quality. The correlations between percent tailless sperm from dilutions and from smears were uniformly highly significant for poor rams and non-significant for the good rams.

Differences between workers, dilution preparations, rams and rams grouped by semen quality were tested by covariance methods as presented

in table 2. There were no differences between workers or preparations for means or for regression coefficients. There were highly significant differences between semen quality groups for both means and regression coefficients. Rams within semen quality groups were not significantly different for either statistic. Analyses within semen quality groups indicated that no important effects were masked by pooling the dissimilar groups. When large percentages of tailless sperm were found in the diluted semen, significantly greater percentages of tailless sperm were present in the corresponding opal blue stained smears. This increase was due to two components, one

TABLE 2. TESTS OF SIGNIFICANCE OF MEANS AND REGRESSION COEFFICIENTS FOR COVARIANCE RELATION OF HEMACYTOMETER DILUTIONS TO OPAL BLUE STAINED SMEARS

Source	For means			For regression coefficients		
	Degrees of freedom	Error of estimate	Mean square	Degrees of freedom	Error of estimate	Mean square
Within workers	90	4183.1452	46.4793	89	4300.2736	45.2839
Between workers	1	0.0808	0.0808	1	152.8716	152.8716
Within preparations	90	4182.3360	46.4704	89	4166.0296	46.8093
Between preparations	1	0.8900	0.8900	1	16.3064	16.3064
Within semen quality	90	3098.8493	34.4316	89	2597.6051	29.1865
Between semen quality	1	1084.3767	1084.3767**	1	501.2442	501.2442**
Within rams & semen quality	88	3071.6445	34.9050	87	2465.3309	28.3371
Between rams & semen quality	2	27.2048	13.6024	2	132.2742	66.1371

** Probability less than .01.

represented by the regression 1.049, which was not significantly different from 1.000, and the other the intercept value of 3.314. The intercept value would make an increase of nearly 6 percent at an observed value of 50 percent for the dilution, on the original scale. Biologically speaking, these statistics indicate that smearing placed a constantly greater strain upon the necks of the sperm than did dilution, thereby causing more to break and form tailless sperm, in semen samples of poor quality. Since smearing created no more tailless sperm than diluting when good samples were used, a weakness of some nature made the necks of sperm in the poor samples susceptible to breakage.

The numbers of tailless sperm were not significantly different between dilutions made with phosphate buffer and dilutions made with chlorazene. The increased numbers of tailless sperm noted by Mercier and Salisbury (1947) were probably due to the physical effects of the process of clearing and staining, rather than to any specific effect of chlorazene upon the necks of the sperm.

It was found, from observation of a drop of semen and a drop of opal blue stain gradually mixing under a cover slip, that sperm which were motile never exhibited any stain in the head and when their motility was lost they invariably stained with both opal blue and eosin. The opal blue stained the post-nuclear cap region and eosin stained the head throughout. Eosin entered first, at the base of the head, and gradually spread to the tip. When poor semen was observed, large proportions of tailless heads were seen which nearly all stained only with eosin. Ten slides each of good and poor semen were reexamined (table 3) and the sperm classified according to whether the heads stained only with eosin or took both stains or neither. The mean number of tailless sperm taking no stain or both stains was not significantly

TABLE 3. STAINING REACTIONS OF SPERM DIFFERENTIATED BY THE USE OF OPAL BLUE AND EOSIN WITH VARIATES TRANSFORMED

Sample number	Good quality semen				Poor quality semen			
	Normal sperm taking—		Tailless sperm taking—		Normal sperm taking—		Tailless sperm taking—	
	No stain or both	Eosin stain only	No stain or both	Eosin stain only	No stain or both	Eosin stain only	No stain or both	Eosin stain only
1	69.73	0	18.44	8.13	33.83	9.98	19.37	47.87
2	80.02	0	9.98	0	42.71	12.92	8.13	43.28
3	84.26	0	0	5.74	9.98	8.13	5.74	75.82
4	67.21	0	9.98	20.27	11.54	11.54	0	73.75
5	75.82	0	11.54	8.13	49.02	8.13	5.74	39.23
6	84.26	0	5.74	0	48.45	5.74	12.92	38.06
7	90.00	0	0	0	43.85	12.92	5.74	42.71
8	74.66	0	12.92	8.13	46.15	8.13	0	42.71
9	75.82	0	14.18	0	35.06	5.74	9.98	52.53
10	81.87	0	5.74	5.74	32.59	0	0	57.42
Mean	78.36	0	8.85	5.61	35.32	8.32	6.76	51.32
Actual %	95.9	0	2.4	0.96	33.4	2.1	1.4	60.9

different between good and poor semen. None of the normal sperm from good semen took only eosin.

Opal blue stained smears were made of semen from the ampulla, tail of the epididymis and head of the epididymis upon autopsy of 384RW and five additional rams not contributing to the previous data. Three rams with good quality semen exhibited practically no tailless sperm at any location. The smears from 384RW and two other rams with defective semen of the same type were very similar in pattern. There was no difference between the head and tail of the epididymis, the tailless sperm constituting less than 10 percent and live and normal sperm being greater than 90 percent. In the ampulla the tailless sperm had increased to 20 percent with a corre-

sponding decrease in live and normal sperm. The final ejaculates contained 60 to 90 percent tailless sperm, and low percentages of live and normal sperm. From counts of 100 tailless sperm, the percent of heads taking both stains or no stain averaged 87 for the epididymis, 8 for the ampulla, and 3 for the ejaculates.

Evidently the sperm generally became tailless after leaving the epididymis. Further experimentation would be necessary to determine whether the unstained tailless sperm in the epididymis were entirely created by the smearing technique, or whether a portion of them were truly tailless. However, the point is not important since the greater proportion of the tailless sperm in ejaculates must have been derived from sperm which appeared live and normal in the epididymis. The results indicate that tailless sperm staining only with eosin should be classified as abnormal. Tailless sperm which take no stain or both eosin and opal blue are probably normal sperm that have been injured in the staining technique.

Summary

The differential features of tailless sperm from a pair of rams with good semen qualities and a pair with poor semen qualities were examined. Counts were made from dilutions in the hemocytometer, using chlorazene and phosphate buffer as the two diluents, and from opal blue stained smears. Differences between means of transformed percentages and analysis of covariance were utilized.

Smearing created no more tailless sperm than diluting if good quality semen was used but if poor quality semen was used about 6 percent additional tailless sperm were produced by smearing when 50 percent were found in the dilution. The percentage of tailless sperm, as measured in the hemocytometer, was practically the same whether chlorazene or phosphate buffer was used as the diluent. Differences between workers, dilution preparations and rams within semen quality groups were not significant by covariance analysis. Differences were highly significant between the pairs of good and poor rams.

In opal blue stained smears of good quality semen, heads of morphologically normal sperm took no stain or both eosin and opal blue, while the tailless sperm were about equally divided between this type and those staining only with eosin. In smears of poor semen, most of the tailless sperm and a small percent of morphologically normal sperm took only eosin. The percentage of tailless sperm which took no stain or stained with both stains was not different between the two qualities of semen.

Stained smears of semen from the epididymis, ampulla and final ejaculates of autopsied rams were examined. No changes in percent tailless sperm

were revealed for three good rams, whereas for three rams with comparably poor semen percentages in the epididymis were similar to the good semen but a definite increase of tailless sperm was shown in the ampulla with the most marked increase occurring between the ampulla and ejaculates. Tailless sperm staining only with eosin, as a percent of all tailless sperm, increased in a corresponding progression from epididymis to ejaculate.

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