APPLICATION OF EOSIN FOR DIFFERENTIAL STAINING OF BUFFALO SPERMATOOA

IV. USE OF OPAL BLUE–EOSIN

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Buffalo semen has received relatively little attention owing to the fact that buffaloes are mainly found in recently developing countries, where artificial insemination is not widely applied. The methods routinely followed in artificial insemination may require to be modified so as to suit the nature and peculiarities of this species.

In the process of semen evaluation, the motility test has a special importance, as fertilization is expected only when there is a certain percentage of active, progressively motile spermatozoa. Differential staining can be used for estimating the percentage of live spermatozoa. Staining with opal blue–eosin mixture has been applied to a certain extent in semen evaluation. Lasley & Bogart (1943) employed this mixture for beef cattle and reported a correlation of +0.41 between the percentage of live spermatozoa and motility, and +0.83 between fertility and the percentage of live spermatozoa. Emik & Sidwell (1947) stained ram semen with a solution containing 12.5% opal blue and 0.5% eosin. However, Schmidt & Jenichen (1952) reported that opal blue–eosin was unsuitable for semen evaluation, particularly in the case of fresh ejaculates. Rao (1957a) stained undiluted buffalo semen for the study of sperm morphology and found that, among the stains giving a blue background, 7% methyl blue and 1:2 to 1:4 dilution of the stock opal blue solution gave the best results. Congo red (2%) was also very good. Rao (1957b) reported that the stock opal blue solution diluted 1:5 is one of the best blue counterstains. Nour El-Din, Omar, Younes & Abboud (1968) and Nour El-Din, Younes, Abboud & Omar (1968) found that, for buffalo semen, yellowish eosin was more efficient than bluish eosin and the optimum concentration of the stain was 1.6 to 1.7%. The present work was carried out to determine the efficiency of opal blue at two levels (12.50% and 6.25%), each combined with 1.70% yellowish eosin, in the process of buffalo semen evaluation. The suitability of opal blue–eosin in comparison with other stain mixtures containing nigrosin, methyl blue and congo red as backgrounds was also determined. Weighed amounts of opal blue and eosin were dissolved in normal saline solution. Mixtures composed of 10% nigrosin, 7% methyl blue and 2% congo red, each combined with 1.70% yellowish eosin, were also prepared. The study included fifteen ejaculates from three buffalo bulls. Motility ratings were made initially on undiluted buffalo

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semen according to the method of Milovanov (1962). Fresh undiluted buffalo semen was used for differential staining. Staining was performed with the five stain mixtures on five smears from each ejaculate, the smears being spread over the whole slide and fixed by air drying. Four fields of view, chosen at random and representing the whole area of the slide were microscopically investigated. The numbers of unstained (live) and stained (dead) spermatozoa were counted in each field.

The mean percentage of motility was 63.00 ± 1.57 . The results of differential staining with each stain mixture are given in Table 1. The background was

TABLE 1
DIFFERENTIAL STAINING OF BUFFALO SEMEN WITH DIFFERENT STAIN MIXTURES

<i>Stain mixture used</i>	<i>No. of unstained sperm.</i>	<i>No. of stained sperm.</i>	<i>Total count of sperm.</i>	<i>% live sperm.</i>
12.50% opal blue-eosin	2462	4608	7070	34.82 ± 0.57
6.25% opal blue-eosin	4701	2479	7180	65.47 ± 0.56
Eosin-nigrosin	4465	2580	7045	63.38 ± 0.57
Methyl blue-eosin	3924	3216	7140	54.96 ± 0.59
Congo red-eosin	3765	3305	7070	53.26 ± 0.59
Total	19317	16188	35505	54.41 ± 0.26

bluish with opal blue and methyl blue mixtures, and reddish with congo red. Nigrosin imparted a dark blackish background, which was better, as it provided a stronger contrast between the background and the spermatozoa. However, Rao (1957a) considered that stains with a blue or bluish violet background were better than nigrosin.

The present results revealed that 6.25% opal blue-eosin mixture was more suitable than 12.50% opal blue-eosin, as it led to a higher percentage of live spermatozoa, corresponding better with the observed motility, and, as reported by Lasley & Bogart (1943), showing a high positive correlation with it ($r = +0.903$). Use of 12.50% opal blue-eosin led to an insignificant positive correlation ($r = +0.194$) with motility. Moreover, by applying the lower concentration of the stain, the differential count was easier and the cytoplasmic droplets were more distinct, appearing as white, unstained areas or as pale blue masses. With the higher concentration, the droplets were very dark and sometimes obscure. This indicates that stain concentration can influence the results of differential staining in buffalo semen. The percentage of live spermatozoa obtained by using 6.25% opal blue-eosin was somewhat higher (2.47) than the observed percentage of motile spermatozoa. This may be attributed to the fact that some spermatozoa were still alive at the moment of staining, but were not motile. The cellular membranes of these spermatozoa presumably kept their impermeability to stains and accordingly were counted as alive.

Our conclusion concerning the value of the opal blue-eosin mixture is not in agreement with Schmidt & Jenichen (1952), who maintained that this mixture was unsuitable for semen evaluation.

For the differentiation of spermatozoa, eosin-nigrosin was found to be more suitable, as it led to a better staining reaction and a percentage of live sperma-

tozoa that coincided more closely with motility, but for the better clarification of cytoplasmic droplets, opal blue-eosin can be used. The other stain mixtures cannot be recommended in the practice of artificial insemination in buffaloes, as they lead to significant variations between estimated percentages of live and motile spermatozoa.

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