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Short Communication

Mountant-induced loss of Shorr-stained blue sperm tails

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Abstract

It has been reported that Shorr staining provides additional morphological information on the motility of spermatozoa in semen, by distinguishing between red and blue flagella. With our routine methods (involving mounting slides) we were unable to confirm these observations. The presence of both red- and blue-coloured sperm tails in Shorr-stained semen smears was apparent, however, if slides were unmounted. Only a very weak association between blue flagellar staining and immotility was observed. Stating whether a mountant was used should be reported.

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1 Introduction

Sperm motility is assessed on living sperm preparations, whereas morphology is performed on fixed, air-dried smears [1]. Useful information may be gained if motile and immotile spermatozoa were distinguishable in semen smears. Haidl *et al.* [2] described one sperm tail defect as abnormal (blue) Shorr staining, related to poor total sperm motility. Men whose sperm motility improved with Kallikrein treatment showed an increase in red sperm tails [3, 4]. The epididymis was implicated since a blue-to-red flagellar change occurred to spermatozoa at the caput-corpus junction [4] where the potential for sperm motility increases. Improvement in caput sperm motility with pentoxifylline, seminal plasma or liposomes was correlated with more red tails [5, 6] and corpus spermatozoa showed parallel increases in motility and red-staining after pentoxyfylline and phospholipid treatment [6]. As the sperm cholesterol/phospholipid ratio decreases upon epididymal maturation [7], lipid components may be eliminated from immature spermatozoa affecting flagellar motility and red staining; indeed, impaired epididymal sperm maturation reflected in asthenozoospermia results in blue sperm-tail staining [8]. These interesting observations, generated by one laboratory, need confirmation. Our laboratory has been unable to confirm the relationship between red-blue tail staining and motility and we sought the reason why.

2 Materials and methods

Air-dried smears of semen from patients attending the Centre of Reproductive Medicine and Andrology of the University Münster, providing a wide range of motile spermatozoa, were examined after Shorr staining for red and blue flagella. The Shorr stain methods used were (1) that given in the World Health Organization

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(WHO) handbook [1] (fixation 75% ethanol 1 min; water 12–15 dips 1 s; Haematoxylin (Merck 9253) 12 min; water 12–15 dips 1 s; ammoniac-alcohol 5 dips 5 s; water 12–15 dips for 1 s; 50% alcohol 5 min; Shorr 3–5 min; 50%, 75%, 96% alcohol each 5 min; 100% ethanol twice 5 min; (2) recipes provided by two laboratories where Shorr staining is routine (e.g. fixation in 100% ethanol for 10 min; 70% ethanol 1 min; Mayer's Hämalaun (Merck 1092490) 10 min 30 s; running water 3 min, 70% ethanol 10 s; 95% ethanol 10 s; Shorr stain (Merck 109275) 1 min; 95% ethanol twice for 10 s; 100% ethanol thrice for 1 s); (3) alterations in the source of stain, relative incubation times in each Shorr stain component, washing time between stains (0.25–24 min) and complete drying after fixation before staining.

Permanent mounting was achieved by treating the slides with Rotisol (Carl Roth GmbH & CO KG, Karlsruhe, Germany) twice for 5 min followed by mounting in Eukitt (Kindler GmbH, Freiburg, Germany). Slides were examined at 1 000 × magnification by brightfield microscopy and oil immersion before and after mounting. Two replicate assessments of 200 cells were made and the mean values accepted when the values were in acceptable agreement [1]. Fixed semen smears were sent to laboratories where Shorr staining was routine and they sent us their Shorr-stained smears.

3 Observations

Ninety to 100% of red-stained sperm flagella were observed in our samples of good and bad motility, irrespective of the protocol employed. However, both red- and blue-tailed spermatozoa were observed at our Centre in semen smears obtained and stained at another centre and also in our samples stained at that centre. The most striking difference between the samples prepared by our Centre and those prepared elsewhere was the absence of coverslip on samples from the centre where blue sperm tails were usual.

Observation of 43 Shorr-stained slides without a coverslip revealed the presence of red- and blue-stained sperm tails, but after coverslipping, significantly fewer blue-stained sperm tails were observed (Figure 1). The mean percentage of blue-tailed spermatozoa in 43 unmounted samples (23.1% ± 2.6% [SEM], median 16.5%), was higher than when mounted (12.0% ± 2.0%, median 8.5%, $P < 0.001$, Wilcoxon Signed Rank Test). The percentage of unmounted blue tails was weakly correlated with immotility (R, 0.219; R^2, 0.048; $y = 0.512x + 0.256$, $n = 45$) (Figure 2); for mounted cells the relationship was weaker (R, 0.221; R^2, 0.049; $y = 0.308x - 1.672$). The percentage of blue-stained flagella in both unmounted and mounted slides was significantly lower (Kruskall-Wallis One Way Analysis of Variance on Ranks, $P < 0.001$) than that of the percentage immotility (44.6% ± 1.1%, median 42.0%).

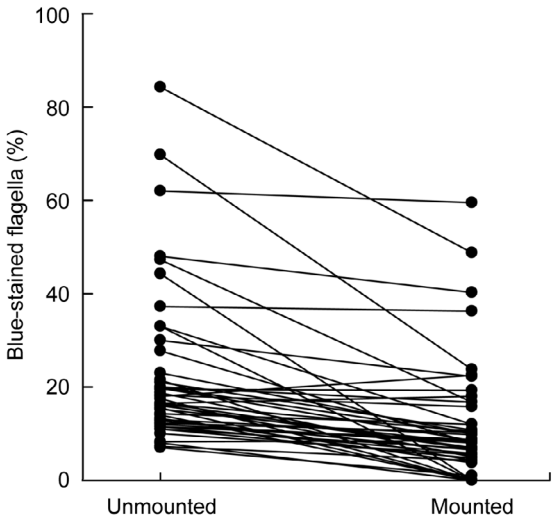

Figure 1. Within-sample paired observations on the percentage of blue-stained sperm flagella (ordinate) assessed in 43 unmounted samples (Unmounted) and in the same samples after mounting (Mounted).

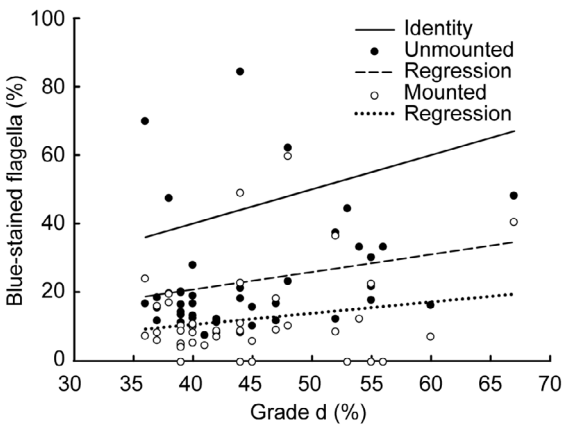

Figure 2. Correlations between the percentage of blue-stained flagella (ordinate) and the percentage of immotile cells (WHO Grade d, determined by technicians: abscissa) in 43 unmounted Shorr-stained semen samples (•) and the same samples after mounting (○).

4 Discussion

Shorr-stained blue sperm tails were observed if the step of mounting the slides was omitted. Although the WHO manual [1] implies the use of cover slips for Shorr-stained semen smears, it is not made explicit. The use of mountants to secure a coverslip on a slide for long-term storage is common-place in histology and such specimens are used for quality control samples, since their properties are thought not to change with time. Their refractive indices (RI) after drying (1.50 –1.55) are similar to those of cells (~1.50) and glass (1.50–1.58) so that together with immersion oil of similar RI (~1.52), they provide the best optical quality by improving resolution and brightness, especially for high magnification assessment of sperm morphology. Mountant use is not normally associated with changes in stained cell colour, but here obvious interference with detecting blue sperm tails was striking. It is not clear whether the clearing agent, or which of the mountant's components (base, an antifade reagent, plasticizer, preservative), was responsible for the interference.

Only a poor relationship between sperm immotility and blue flagellar staining was evident here, in contrast with the excellent relationship between the two (R, 0.991, R^2, 0.983, $y = 0.987x + 7.092$, $n = 18$) reported by Haidl *et al.* [4] (his Table 2). As epididymal samples, they differ markedly in nature from ejaculated samples where accessory gland secretions may influence sperm tail staining. Studies to compare different clearing agents and mountants should be performed to determine if this can be confirmed and whether it is a general or reagent-specific phenomenon.

5 Conclusion

If the Shorr stain is used for assessing sperm flagellar colouration, stating whether a mountant was used has to be reported.

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