

Methylene blue reduction test in semen analysis of White Pekin ducks

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The low fertility and hatchability of duck eggs to produce day-old ducklings is the real problem for non-availability of required germplasm to propagate in extensive way unlike chicken. Sophisticated measurements of sperm mobility are also taken more rapidly with the computer-assisted sperm analysis (CASA) (Tardif *et al.* 1998). Metabolic tests such as fructolysis and oxygen consumption are important measures of sperm function (Bratton *et al.* 1956). However, these assays are highly correlated with a simple test that reflects sperm metabolism such as the reduction of methylene blue (MBRT) to the reduced colourless form by accepting 2 hydrogen atoms during sperm metabolism (VanDemark *et al.* 1945). The present study was carried out with the intention to find out an efficient semen quality test for wider application in duck breeding programmes.

White Pekin drakes (10) of 30–45 weeks of age were randomly selected and were individually caged and maintained under standard optimum management practices. Semen samples (32) were collected at 2 days interval by adopting the abdominal massage method (Burrows and Quinn 1936). Routine micro and macroscopic semen quality tests were carried out immediately after collection. Colour (scale 1 to 5), pH, mass motility (scale 0 to 5) (Pistenma *et al.* 1971), individual motility (under microscope by standard procedure described by Zemjanis 1970), volume (by a tuberculin syringe), concentration (by haemocytometer method as described by Jones and Wilson 1967), number of spermatozoa/ejaculation (multiplying concentration with volume), livability (using Eosin-Nigrosin stain as described by Saxena 2000), sperm abnormality (using Giemsa staining technique as mentioned by Sharma 1995) were estimated along with methylene blue reduction time recorded (Herman and Madden 1953).

A good amount (2 to 4 ml) of clear and thin discharge was usually encountered before actual semen discharge in ducks while collecting semen. The mean±standard error

Table 1. Routine seminal parameters of White Pekin Duck

Seminal attributes	Mean±SE	Range
Colour (1–5 scale)	3.41±0.13	2–4
pH	7.58±0.03	7.3–7.8
Volume (ml)	0.83±0.39	0.4–2.1
Mass activity (Scale 1–5)	3.41±0.13	2–4
Individual motility (%)	87.25±0.73	78–95
Concentration (×10 ⁹ /ml)	1.09±0.10	0.44–2.8
Number of spermatozoa/ejaculation (× 10 ⁹)	0.79±0.05	0.32–1.2
Live sperm (%)	92.59±0.55	84–98
Total sperm abnormality (%)	8.90±0.46	5–15
MBRT (min)	10.03±0.24	8–13

of the different physical parameters are presented in Table 1.

Since it is not feasible to study all the seminal parameters to throw light on the quality of semen, it is relevant to know the possible correlations among different seminal parameters, so that the semen quality can well be judged by estimating few parameters. In this context, the correlation among different seminal parameters estimated is presented in Table 2.

Colour: The natural colour of drake's semen is almost creamy white. The discolouration of semen was mostly due to contamination with excreta and glandular secretions which passes through the surface epithelium of cloaca during semen collection. Majority of the semen samples from White Pekin drakes were medium thick in consistency and this was in agreement with the findings of Nair (2003) and Cyriac *et al.* (2013). Highly significant positive correlation between concentration and colour was recorded, which indicated that colour of semen samples is largely governed by the concentration of semen.

pH: The pH of the freshly collected duck semen did not show correlation with other parameters and it was found to be slightly alkaline, which supports the findings of Cyriac *et al.* (2013). Contrary to this, Etuk *et al.* (2006) reported a higher pH in Muscovy under different management systems. The pH of semen is not a reliable semen quality test as soon after collection, there is a sharp decline in pH due to production of increased amount of lactic acid released from

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Table 2. Correlation among different seminal parameters of white Pekin duck semen

Seminal parameters	2	3	4	5	6	7	8	9	10
pH	-0.004	0.238	-0.170	0.121	-0.160	-0.289	0.191	0.086	0.146
Mass motility (scale 1-4)		-0.179	-0.102	0.225	0.319	0.367*	-0.069	-0.136	-0.046
Individual motility (%)			0.231	-0.207	-0.142	0.003	0.779**	-0.237	0.375*
Volume (ml)				-0.676**	-0.556**	0.294	0.449**	-0.416*	0.296
Colour (scale 1-5)					0.848**	0.383*	-0.330	0.235	-0.564**
Concentration ($\times 10^9$ /ml)						0.541**	-0.298	0.068	-0.557**
No. of spermatozoa/ejaculation ($\times 10^9$)							0.450	-0.363*	-0.382*
Livability (%)								-0.422*	0.418*
Sperm abnormality (%)									0.055
MBRT (min)									

* Significant ($P < 0.05$); ** highly significant ($P < 0.01$).

fructolysis. Besides, false results may be encountered due to uric acid crystals from urinal contamination.

Volume: Highly significant negative correlation of semen volume with colour and concentration of semen were also recorded. The difference in volume of semen harvested was due to the larger amount of accessory fluid secretion from vasa deferentia and fluid from the ejaculatory groove region.

Mass motility: The reported mean mass motility score of spermatozoa was similar to the findings of Cyriac *et al.* (2013), who recorded an initial motility of 3.38 in White Pekin drakes. There was no marked correlation of mass motility with other semen parameters. Though the motility estimation can be used to detect gross differences in semen quality, they have limited value in determining small fertility differences (Nair 2003). The presence of very long tails and high sperm concentration also acts as limiting factor.

Individual motility: The finding of motility per cent was similar to those of Domyati drakes as reported by Ghonim *et al.* (2010). A highly positive correlation between livability and individual motility was obvious for which, it is considered more effective semen quality test.

Concentration and total number of spermatozoa per ejaculation: A higher concentration of semen (3.03 billions/ml) has been reported by Cyriac *et al.* (2013) than the present findings. A highly negative correlation was reported between concentration of semen and number of spermatozoa per ejaculation with MBRT and volume. A wide variation in the concentration of spermatozoa in duck semen is attributed to the different methods adopted for estimation of concentration and possible contamination with the transparent fluid at the time of semen collection.

Livability: The present observation of live percent of sperms is comparable to the findings of Ghonim *et al.* (2009) in Domyati drakes but little lower (96.45%) than that found by Cyriac *et al.* (2013). It is highly correlated with volume and individual motility, giving a better information about the semen quality. But livability is also influenced by different factors like method, time and season of collection.

Sperm abnormality: The present observation of sperm finds the support of Gvaryahu *et al.* (1984), who reported similar scores in Muscovy drakes but are lower as compared to that in Domyati drakes, (Ghonim *et al.* 2009).

MBRT: The overall mean time to decolourise methylene blue in the white Pekin Ducks was higher compared to the reports of Nair (2003) and Cyriac *et al.* (2013). Among all seminal parameters studied in the present investigation methylene blue reduction test was reported to be highly correlated with other parameters. The mean methylene blue reduction time was negatively correlated with mean spermatozoa concentration and colour of semen samples but is positively correlated with the number of live spermatozoa. In the present report it was one of the few statistically significant semen characteristics observed. Earlier VanDemark *et al.* (1945) also had shown that the MBRT was highly correlated with sperm concentration (-0.81), the percentage of motile sperm (-0.63), glucose loss (-0.78), and lactic acid gain (-0.75) in bull. So, it is regarded as an important mean to determine the metabolic activity of spermatozoa.

MBRT is reported to be highly correlated with spermatozoa concentration, livability and semen colour. It is a test that reflects metabolic activity of the sperm cell, such as ATP, and is simpler to run. Therefore, methylene blue reduction test was found to be a useful and reliable test to estimate the semen quality in duck.

SUMMARY

This study was undertaken to investigate the relationship between methylene-blue reduction time (MBRT) and other semen quality parameters in duck by analysis of correlation coefficients. Semen ejaculates (32), collected at 2 days interval from 10 randomly selected drakes, were examined for different seminal parameters like pH, volume, colour, mass activity, individual motility, livability, concentration, number of spermatozoa per ejaculate, total sperm abnormality per cent and MBRT. The overall mean time to decolourise methylene blue in the white Pekin ducks was 10.03 ± 0.24 min with a range from 8 to 13 min. MBRT was negatively correlated with mean spermatozoa concentration, number of spermatozoa per ejaculation and colour of semen samples but positively correlated with individual motility and livability. High correlation of MBRT with seminal parameters indicated its practical utility in monitoring semen characteristics.

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