ASSESSMENT OF BOVINE SEMEN QUALITY BY RESAZURIN REDUCTION TEST

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ABSTRACT

A study was conducted to assess the semen quality using Resazurin Reduction Test (RRT) in Jersey and Jersey cross bulls. A total 36 fresh ejaculates were collected for evaluation RRT, sperm motility, acrosomal integrity and sperm concentration. The mean value of RRT in bulls was 15.77±0.85 minutes.Statistical analysis revealed that RRT was highly significantly ($p<0.01$) and positively correlated with sperm concentration (r=0.769), sperm motility(r=0.858) and acrosomal integrity (r=0.515), hence it may be helpful in evaluating the quality of semen in bulls.

KEY WORDS: Bull Semen, Resazurin reduction test, Semen quality, Correlations.

INTRODUCTION

Semen evaluation is the most important part in predicting fertility of domestic animals. Due to the complexity of the fertilization process, a single semen evaluation test is not able to predict fertility (Dhurvey *et al*., 2012).Therefore, examination of semen based on various parameters like gross examination, viability, *in vitro* fertility, metabolic activity and bacterial contamination etc. can give a fair assessment of its fertilizing potential (Shukla, 2011). Metabolic tests such as fructolysis and oxygen consumption are important measures of sperm function, but are not done routinely because of their complexity (WHO.1992). The resazurin reduction test (RRT) depends on the ability of metabolically active cells to reduce the resazurin to resorufin and thus it can be used to monitor the sperm cell viability (Erb 1950). They also found a significant negative correlation between RRT and fertility (*r*=-0.17) of bulls, but not MBRT with fertility.So the present study was designed to assess semen quality of bulls using RRT.

MATERIALS AND METHODS

Twelve healthy bulls (Jersey and its cross) belonging to Frozen Semen Laboratory, Nagpur were selected for the present study. Semen was collected from these bulls twice a week with an artificial vagina (40 cm long and 6.5 cm in diameter) maintained at 42-45°C. Total 36 fresh ejaculates (3 ejaculates from each bull) were collected and evaluated for various seminal parameters. Concentration of spermatozoa (million/ml) was determined by bovine photometer No. 1062 (Photometer, IMV Technologies, France). The per cent progressive motile spermatozoa was assessed as described by Ray and Ghosh (2013) and acrosome integrity by Giemsa staining (Watson, 1975).

Resazurin solution was prepared by mixing 50 mg Resazurin (7-hydroxy-3H, Phenoxin-3, one-10-oxide) in 100 ml of 0.9% distilled water. 0.2 ml of fresh semen was taken in a test tube and 0.1 ml resazurin solution was added. This solution was layered with small quantity of mineral oil. Test tube was kept in incubator set at 45°C for colour change. The time required for colour change from dark blue to violet, violet to pink, and pink to white was noted in minutes.The SAS software (SAS enterprise guide 4.3) was used to analyse Pearson correlation coefficients among RRT, sperm motility, acrosomal integrity and sperm concentration.

RESULTS AND DISCUSSION

The average time for RRT in bulls was15.77±0.85minutes. These observations nearly corroborated

with the reports of with Dart *et al*. (1994), who recorded <15 min for reduction of resazurin from blue to white in Limousine bulls. Similarly, Pathak *et al.* (1989) recorded time interval in crossbred bull for reduction of resazurine from pink to white (7.0 to 15 min), which is found to be similar with the present findings. The time required for reduction of resazurin is dependent on the quality of semen in terms of concentration and percentage of actively motile spermatozoa (Erb and Ehlers, 1950). The mechanism of resazurin reduction has been postulated to be due to oxygen consumption through metabolism or mitochondrial enzymes, oxido-reductases (Zhang *et al.,* 2004). Therefore, measurement of resazurin reduction is an indication of mitochondrial function. The RRT is a better metabolic assay than measuring ATP (Mahmud *et al*., 1994).

The study revealed that RRT was highly significantly (P<0.01) and positively correlated with sperm concentration (r=0.769), sperm motility (r=0.858) and acrosomal integrity (r=0.515). Further, sperm motility and sperm concentration were highly significantly (P<0.01) and positively correlated, but the acrosomal integrity had no correlation with sperm motility and sperm concentration.

The present findings were in close agreement with Dart *et al*. (1994), Zrimsek *et al*. (2004) and El- Battaway and El-Nattat (2013), who reported that RRT was highly correlated with sperm concentration and percent motile sperm in boar,bull and rabbit semen, respectively. Fuse *et al*. (1993) reported that the RRT was more useful than most other criteria in evaluating fertility potential of human semen, being highly correlated with the fertility. The RRT is a rapid method for estimating fertilizing capacity of bulls(Erb and Ehlers, 1950) and could distinguish between semen samples in which sperm produced varying amounts of reactive oxygen species that cause lipid peroxidation of sperm membrane leading to poor sperm function (Zalata *et al*., 1998).The assessment of the reduction of resazurin from blue to pink and from blue to white helps in identification of high fertility potential sample in bovine semen (Dart *et al*., 1994).

In conclusion the resazurin reduction test was found to be a reliable method in evaluating bovine semen viability and can be used successfully, especially in routine analysis.

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DYSTOCIA AND ABORTION IN A MARE - A CASE REPORT

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Spontaneous abortion is a common cause of reproductive failure in domestic animals. It is highest in cattle, particularly in dairy cow, but also in sheep and horses (Benirschke, 2012). In mares, abortion is defined as the expulsion of the fetus before it reaches the 300-days gestation period. A variety of medical reasons including infectious agents, chemicals, hormonal and nutritional problems and twinning can cause the loss of the foetus. Non-infectious causes of abortion are more common than infectious causes (Robinson, 2009). Lack of feed or poor quality feed, along with poor body condition can result in abortion. Dystocia is a significant event in equine reproduction. In one study, dystocia were the second most common cause of equine pregnancy loss, accounting for 19% of losses (Giles *et al.,* 1993). Every equine dystocia is an emergency and requires clinical skill and quick decisions (Norton *et al*., 2007).The present report describes a rare case of dystocia in mare associated with partial abortion.

CASE HISTORY, OBSERVATIONS AND TREATMENT

A 4 years old mare reared on conventional grazing system and paddy straw only was presented to the TVCC of the College in Nagpur at about 9th month pregnancy with the symptom of anorexia and straining. The temperature of mare was recorded as 103.6°F. Per-vaginal examination revealed incompletely dilated cervix and presence of the foetus in dorso-sacral position with vertex presentation. Only the nose of the foetus was caught on the brim of the pelvis with the fore head entering the pelvic inlet. The mare wasfound negative for both bacteria (*Brucella spp.*) and parasites when blood sample was examined. Treatment and fluid therapy (Dextrose 10%) was administered along with injection Tribivet -10 ml (Intas), inj. Melonex plus -15 ml(Intas), and parenteral antibiotic (Oxytertracycline- 30 ml). By the application of retropulsion and then forceful traction in synchrony with the efforts of the mare using the obstetrical ropes around the foal's pastern, the dead, partially putrefied and foul smelling foal was removed.

DISCUSSION

On the basis of the history and report of clinical pathology, mare was not suffering from any bacterial or parasitic diseases. As no balanced ration was provided to the mare before or during the gestation period, the death of the foal might have occurred due to nutritional deficiency. Dystocia can be caused by either maternal or foetal problems. The majority of cases that are seen are caused by foetal problems, mainly mal-presentation, abnormal position or posture of the foal. One of the most common postural abnormalities is a simple retained forelimb or head and neck deviation (Mc Cue and Ferris, 2012). Victoria (2014) reported that 50% of severe dystocia were due to mal-presentation of the foal, 17% were due to contraction of the foal limbs, 8% due to other conformational abnormalities, 6% due to foetal oversize and 19% due to abnormalities or disease affecting the mare. Norton *et al.* (2007) reported that the survival rate of emergency case of dystocia in foal is low. According to Dube (2012) and Higgis and Synder (2013) dystocia in mare occurs due to foetal deformity and nutritional and energy deficiencies may be responsible for abortion in mare. Experimentally, nutritional deficiencies in pregnant females produced abortions (Benirschke, 2012). However, the present finding contradicted. Wright (2009) who stated that nutritional deficiencies have not been associated with abortion in mares.

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