**Real time biomarkers affecting fertility of Bulgarian Murrah buffalo depending on age and season**

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Oxidative stress has a negative effect on the reproductive process in mammals. It is therefore desirable to determine and control their oxidative/antioxidant status in order to prevent pathological changes leading to problems in fertilization, embryonic development and survival of the offspring. The danger of oxidative stress stems from the fact that it is not proven by clinical symptoms. The condition is diagnosed by biomarkers of oxidative stress, such as the levels of a number of products of radical damage to cellular structures, lipids, proteins, nucleic acids and the activities of antioxidant enzymes and others.

**Aim:** The aim of the presented study was: 1. To measure some real time biomarkers of radical formation - oxygen species (ROS), ascorbate radicals (Asc*), nitric oxide radicals (NO•); 2. To discuss the influence of the measured real time biomarkers on fertility of buffaloes and buffalo heifers of Bulgarian Murra breed during non-breeding and breeding season.

**Materials and Methods:** The study included 24 multiple-born buffaloes> 40 days postpartum and 30 buffalo heifers of the Bulgarian Murra breed. The animals were kept on the buffalo farm of the Agricultural Institute, Shumen (Northeastern Bulgaria, latitude: 43.28N, longitude: 26.93E). The ration included concentrated fodder for lactating buffaloes, beer porridge, alfalfa hay, straw, and constant access to water. The buffaloes weighed from 460 kg to 650 kg, aged from 2 to 6 years and lactating milk 1800 - 2100 kg with daily double milking. The buffalo heifers had a body weight of 450 - 470 kg and an age of 820 - 840 days. The ration consisted of concentrated fodder, alfalfa hay, straw and constant access to water. Group of buffaloes (n = 24), non-breeding (spring and summer) season (n = 8) and breeding season (autumn and winter) (n = 16); Group of buffalo heifers (n = 30) non-breeding season (n = 17) and breeding season (n = 13).

Increased oxidative stress was registered in buffalo heifers during the breeding season, compared to buffaloes in both seasons through a statistically significant increase in ROS products in blood serum. The elevated levels of ROS, ascorbate radicals, and nitric oxide were reported in non-pregnant buffalo heifers, but without a statistically significant difference. However, buffalo heifers were found to have better fertility, probably due to more active antioxidant protection as an adaptive mechanism against oxidative stress.

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