A Cross-species Comparison of a Specimen Collection Container Designed to Harvest Oxygen Radical Species

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Abstract

This study was designed to compare a novel specimen collection container designed to harvest oxygen radical species (OXS) with standard methods. The specimen collection container designed to harvest OXS was developed at the Texas Tech University (TTU) Health Sciences Center. Sixteen normal human subjects were enrolled in this study and their blood was drawn into the novel specimen collection container designed to harvest OXS and into standard EDTA and Na2EDTA collection tubes. Both the novel specimen collection container designed to harvest OXS and the standard EDTA and Na2EDTA collection tubes were analyzed for their capability to reduce OXS generation, reduce cell damage, and preserve cell viability.

Introduction

It is well documented that high free-oxygen radical concentration within the fat environment is associated with the development of various diseases such as cancer, diabetes, and cardiovascular disease. Therefore, it is important to investigate the mechanisms that lead to impaired oxygen radicals. Previous research from this laboratory resulted in creating a new collection system, referred to as the Protex (Reproductive Sciences, Inc., Sante, TX). The system provides a superior way of preparing collection environment for minimal OXS generation and effective cell preservation. Through this study, we aimed to investigate the capability of the novel specimen collection container designed to harvest OXS in comparison to the standard EDTA and Na2EDTA collection tubes.

Materials and Methods

Ten donors were recruited with normal serum parameters at testing using the World Health Organization version 6 parameters for a normal serum analysis. Each donor then supplied 4 samples, a minimum of 2 ml per sample from a minimum of 8 days apart. The treatments were as follows:

1. A standard serum cup with 1 ml of Fuji Fine-Multipurpose Handling Media (MHM)
2. An original Protex, with 1 ml of MHM
3. A simple with 1 ml of MHM

Donors were assigned randomly to which treatment they started with i.e., if assigned to start with treatment 1 then proceed to 1, and finished with 3. Serum samples were collected into the novel specimen collection container designed to harvest OXS. The sample was allowed 15-30 minutes to equilibrate before undergoing a serum analysis. The serum analysis consisted of nuclear volume measurement (Kihara et al. 2018), red cell count, average platelet count, average platelet volume, hemoglobin, red cell count, and platelet count.

Results

1. As expected all serum parameters tended to decrease over time due to the storage device.
2. Data from the human samples suggested the Protex and Protex+ maintained equal or better motility over the analyzed time period compared to the original Protex. See Figure 1. While none of the individual motion parameters in the human trial where better in the Protex+ versus the control, collective all were higher in the Protex+, Table 1.
3. Similar results were seen in the equine where the Protex+ maintained equal or better motility and rapid cell movement 12 hrs while stored under suboptimal conditions (Figures 2 and Figure 5: p < 0.05).
4. Similarly, equine samples collected and stored in the Protex+ collectively had better motion parameters than the Protex or baby bottle control.

Discussion

1. It is important to note these experiments were conducted in environments meant to induce accelerated free oxygen radical development.
2. Data from these trials support previous work that suggests the Protex is a better system for semen collection.
3. Further, data suggest the Protex+ with its redesign to include antioxidant properties, is superior to either traditional methods or the conditions under extreme oxidative stress conditions.
4. Further study in a more controlled environment will be needed to verify these observations.

Materials and Methods Cont

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Figure 1. Normalized mobility and rapidity of controlled bovine samples incubated in a Standard Specimen Cup (SSC), an original Protex, and in a Protex+ for at least 6 hrs (p < 0.0001). Individual means with different superscripts are different within the same point.

Figure 2. A comparison of normalized mobility in a preliminary trial of a given bovine sample incubated in a Standard Specimen Cup (SSC) and a Protex+ to show the unchanged free oxygen radicals in a sport environment:

Figure 3. A comparison of normalized mobility in a preliminary trial of a given bovine sample incubated in a Standard Specimen Cup (SSC) and a Protex+ to show the unchanged free oxygen radicals in a sport environment.

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