The Orthopaedic Research Center consists of the Gail Holmes Equine Orthopaedic Center (11,000 square feet), the Orthopaedic Research Laboratories (a total of eight laboratories with separate biochemistry, molecular biology, histology and tissue culture – laboratory space over 4,000 square feet), an equine MRI Center (with a 1 Tesla extremity scanner) and a Gait Analysis Lab for both horses and dogs with full kinetic and kinematic capabilities.

ORC Facility Details:

1. ***Clinical:*** Immediately adjacent to the ORL is the Gail Holmes Equine Orthopaedic Research Center which contains an equine surgery suite. Equipment for radiography is available at the Center which also houses a high speed treadmill for controlled exercise and has 32 stalls for horses. The Veterinary Teaching Hospital (VTH) is within 400 yards of the ORL and contains equipment available for nuclear scintigraphy and computed tomography. Between the Gail Holmes Equine Orthopaedic Center and the Orthopaedic Research laboratory is a Magnetic Resonance Imaging (MRI) Center which houses a high-field extremity scanner able to image the extremities of live horses. These three buildings make up the Orthopaedic Research Center with a total area of 17,000 square feet.

2. ***Laboratory:*** The newly renovated and expanded Orthopaedic Research laboratory (ORL) at Colorado State University (CSU) contains separate biochemistry, molecular biology, histology and tissue culture laboratory space (over 4000 square feet of total laboratory space). There are 4 laboratory technicians dedicated to the ORL. Additionally in the same building is a complete biomedical engineering laboratory.

3. ***Animal:*** CSU horses are routinely used by the ORC for the study of musculoskeletal diseases. There is housing for 36 horses at the Gail Holmes Equine Orthopaedic Research Center and animal care technicians experienced in horse care are employed by the ORC to manage these animals. The animal care is closely monitored by the Animal Care and Use Committee at CSU.

   Laboratory Animal Resources (LAR) unit at Colorado State University (facility is accredited by AALAC and conform to the NIH policy on Humane Care and Use of Laboratory Animals) is located on main campus a half mile from the ORC. The ORC also has a full-time business manager, laboratory manager and full-time receptionist.

4. ***Computer:*** The ORC currently houses 9 desktop computers for use by all personnel. One computer is dedicated to the histomorphometry system (Olympus IX70 fluorescent microscope and Bioquant BQ- TCW98 morphometry program) and one is dedicated to the ASI Prism 7700 sequence detection system. Multiple data ports allow network connectivity for all computers. Most of the computers have statistical analysis (SAS 7.0), word processing (Word) and spreadsheet (Excel) packages installed.

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5. ***Office:*** All necessary office space is available for researchers and administrative staff at the ORC. The ORC also has a full-time business manager and full-time receptionist.

6. ***Laboratory:*** The newly renovated and expanded Orthopaedic Research Laboratory (ORL), contained within the Gail Holmes Equine Orthopaedic Research Center (ORC) at Colorado State University, occupies over 4000 square feet of total laboratory space. The ORL contains separate biochemistry and molecular biology Laboratory space; three tissue culture annexes, and a radioisotope area. There are four laboratory technicians dedicated to the ORL. In the same building is the new Biomedical Engineering laboratory supplying histological tissue processing, image analysis, and biomechanical testing capabilities. The Biomedical Engineering laboratory also contains a third tissue culture annex.

Laboratory Applications Include:

1. Biomarker Analysis

- Fully equipped to run any commercially available absorbance or fluorescence biomarker immunoassay in 96 or 384-well plate format. Using Molecular Devices SpecraMax 384 plus, microplate absorbance/transmittance reader, as well as a Gemini-XS Fluorometer.
- Extensive experience with the following biomarker assays:
  - Detection of Cartilage Markers:
    - **Alcian Blue:** Standardize measurement of ^{35}S labeled proteoglycan complexes.
    - **C1,C2:** An assay to standardize the measurement of Types I and II collagen degradation.
    - **CPII:** An assay to measure type II collagen carboxy propeptide (C-propeptide).
    - **CS-846:** Measurement of Aggrecan Chondroitin Sulfate 846 Epitope.
    - **Eq. Col 2 ¾ (CEQ):** An assay to quantify equine specific Type II collagen, which has also been proven to work with canine fluid.
    - **GAG DMMB:** An assay for standardized measurement of glycosaminoglycans in biological fluids and/or tissues.
    - **Prolagen-C:** Measurement of C-Terminal propeptide Type-I collagen.
    - **Pyd Assay:** An assay to standardize measurement of pyridinoline crosslinks in serum and urine.
    - **Pyrilinks-D**: To standardize measurement of deoxypyridinoline crosslinks in urine.

- **TCA:** Assay to measure 3H content in media or cartilage digested samples.
- **YKL-40:** Assay for measurement of YKL-40, human cartilage glycoprotein 39, in serum.

- Detection of Bone Markers:
  - **C1,2C:** An assay to standardize measurement of Type I and II collagens (378 assay, MMP1 and MMP13).
  - **Metra™ BAP**: Quantification of bone-specific alkaline phosphatase in serum and synovial fluid samples.
  - **Metra™ Osteocalcin EIA:** An enzyme immunoassay for the quantification of intact (de novo) osteocalcin.
  - **Serum Cross Laps® (CTX):** Assay for the quantification of degradation products of C-terminal telopeptides of Type-I collagen in serum and plasma.
- Cytokine Assays:
  - **HIL-1ra:** To standardize the measurement of interleukin 1 receptor antagonist concentrations in cell culture supernatant, serum and plasma.
  - **IGF:** To standardize the measurement of Insulin-like Growth Factor in Serum, Cell culture and plasma.
  - **TGF-β:** An assay to quantify measurement of Transforming Growth Factor-beta in serum, cell culture supernatant, plasma and urine.
  - **TNF-alpha:** An assay to quantify levels of Tumor Necrosis Factor-alpha in serum, plasma, synovial fluid and cell culture supernatant
  - **IL-10:** An assay to quantify levels of Interleukin-10 in serum, plasma and cell culture supernatant.
  - **PDGF-BB:** An assay to quantify levels of Platelet-Derived Growth Factor-BB subunit in serum, plasma and cell culture supernatant.
  - **PGE2:** An assay to quantify levels of Prostaglandin E2 in serum, plasma, synovial fluid, cell culture supernatant and urine.
- Pre-assay sample processing including: papain, hyaluronidase and collogenase digestion as well as chromatography extraction of synovial fluid, serum and tissues.
- Western, Southern, and Northern Blotting
- Many other assays available. Please inquire.

2. Biomechanical Testing

- Displacement control testing for compressive, tension and shear material properties
- Tissue explants or cell-seeded scaffolds
- Light to moderate load cells are suitable for testing small tissue explants or cell-seeded scaffolds

3. ***Molecular Biology***
    - Evaluation of metabolic activity in living tissues
        - Radiolabel protocols available
    - GeneChip® Microarray Analysis
        - Complete Affymetrix GeneChip® 3000 scanner, fluidics 450 and hybridization system.
    - Real Time PCR Analysis
        - ABI Prism® 7000 Sequence Detection System
        - Optimization of PCR Primers
    - RNA/DNA Extractions/Isolations
        - cDNA synthesis from RNA
        - RNA from cells, tissue or whole blood
        - Primer and probe design
        - Gel extraction and purification
        - Purification of plasmid DNA
        - PCR amplification
    - Isolation of Synoviocytes, Chondrocytes, and Tenocytes
        - Cell culture expansion of freshly collected cells
    - Culturing of Mesenchymal Stem Cells (bone-marrow derived or fat-derived)
        - Cell culture expansion of bone-marrow derived or adipose-derived cells, including three-dimensional culturing for clinical use
    - Adenoviral Vector construction and cell transfection
        - The development of adenoviral vectors for the delivery of genes into cells
4. ***Histology Services***
    - Decalcified tissue histology
    - Immunohistochemistry
    - Paraffin and frozen Sectioning and staining of paraffin embedded samples
    - Histomorphometric analysis