* 1. Gently invert the tube before centrifugation. Centrifuge the tube at 2100 gfor 7 min. Remove the supernatant and re-suspend the pellet with 3 mL of expansion media.
     1. Centrifuge the tube at 2100 g for 7 min.Remove the supernatant and add 1 mL of expansion media.

**Characterization of ovarian MSC *in vitro***

The goal of MSC characterization is to ensure that isolated cells conform to standard MSC criteria. These include adherence to plastic, positive detection of of mesenchymal surface markers and absence of hematopoietic surface markers, as well as the capacity to undergo mesodermal differentiation.

As shown in **Figure 3**, ovarian derived cells were adherent to the plastic and exhibited fibroblastic-like morphology, fulfilling the first criterion that defines MSC. Moreover, the isolated cells showed the expression of transcripts for the canine MSC markers CD44, CD90, and CD105. In addition, MSC-specific cell surface antigens CD90 and CD44 were detected by early passage FACS analysis and immunocytochemistry. Absence of the markers CD45 and CD34, which are hematopoietic-specific cell surface antigens, was also confirmed by the same techniques(**Figure 4**). This further confirmed that the ovary contains cells expressing an MSC-specific phenotype9. The antibodies that were used in this experiment for canine MSC characterization are listed in the **Table of Materials**.

The next step in the characterization of MSC is to attain proof of their ability to differentiate into mesodermal lineages. After 30 days of exposure and culture in lineage-specific differentiation protocols, staining was performed to identify commitment to different lineages (**Figure 5A**). Commitment to the osteogenic lineage was confirmed through Von Kossa staining, which identified calcium deposits. Safranin O proteoglycan staining confirmed chondrogenic commitment, and Oil Red O lipid staining confirmed adipogenic commitment Continuing the investigation of the differentiability of these cells, they were able to undergo differentiation into the ectodermic lineage, as shown by immunostaning for two neuroectodermal stem cell markers: β-Tubulin and Nestin, following exposure of ovary-derived MSCs for to the neuronal lineage differentiation protocol for 10 days (**Figure 5C**). Transcripts for SOX17 and CD184, as well as cell morphologies specific to the endodermal lineage, were observed after exposure to specific differentiation protocols for 5 days **Figure 5B**). Finally, after 14 days of exposure of ovarian derived MSC to germ cell induction media, OCT 4 and DDX4 were identified (**Figure 5D**). Together, these results support a robust and diverse capacity of differentiation for MSC derived from ovarian tissue, when exposed to specific differentiation protocols9.