1. PURPOSE

The purpose of this SOP is to describe the collection of endometrial cells for studies investigating the uterine environment at cellular and molecular level.

Endometrial cell collection by cytobrush is a minimally invasive technique, similar to artificial insemination (AI), which allows for evaluation of the molecular phenotype of the endometrium and uterine environment. It has been well demonstrated\(^1,\)\(^2,\)\(^3\) that the collected cells are representative of the dynamic changes that occur during different physiological and pathological conditions of the uterus. Veterinarians for cytological diagnosis of subclinical endometritis also use the technique. Therefore, it represents a valuable tool in basic and applied studies related to fertility in cows.

2. RESPONSIBILITY

Principal Investigator (PI) and their research staff, farm staff, veterinary staff and all qualified personnel who perform or assist with Endometrial Cell Collection by Cytobrush Technique in Cattle.

3. MATERIALS

3.1 Personnel Protective Equipment (coverall, latex/ nitrile gloves, long-sleeve plastic gloves, CSA-approved steel-toe boots, etc.)
3.2 Halter
3.3 Lubricant
3.4 Lidocaine 2%
3.5 21G (4 cm) needles
3.6 5 mL syringes
3.7 Warm water and antiseptic soap (e.g. Endure 400)
3.8 Paper towels,
3.9 Sprayer with iodine solution
3.10 Conventional Artificial Insemination (AI) gun
3.11 Disposable Artificial Insemination (AI) sheath
3.12 Sanitary sheath for AI gun
3.13 Collection tubes (15 ml and 50 ml)

4. GENERAL

4.1 It has been demonstrated that four collections, from days 10, 13, 15 and 19 of the estrous cycle, did not affect the fertility of the cows inseminated at the subsequent estrus\(^1\).

5. PRECAUTIONS

5.1 Monitor for any bleeding in the rectum. Remove the hand in such case.

5.2 Animals will resist insertion of the hand. Do not force, but firmly hold it in place for a few seconds. Continue insertion once the rectal muscles relax.

5.3 The scanning procedure must not exceed a 10 minute duration to avoid inflammation of rectal wall.
5.4 Monitor the animal for signs of pain (e.g. hunching back, twitching of abdominal muscle, kicking, etc.). Slow down or stop depending on the intensity of animal's reaction.

6. **PROCEDURE**

6.1 Restrain the cow in the chute in such a way that it cannot back up.

6.2 Induce caudal epidural anesthesia with 5–10 ml lidocaine, to effect. Refer to SOP DC-701: Substance Administration in Dairy Cattle.

6.3 Make sure the rigidity of the tail base is reduced to confirm successful induction of anesthesia.

6.4 Carefully empty the rectum using lubricated and gloved hand.

6.5 Tie the cow's tail to expose the perineum.

6.6 Don clean gloves.

6.7 Gently wash the perineum with warm water and antiseptic soap (e.g. Endure 400) and wipe with paper towels.

6.8 Spray the perineum with iodine solution and wipe with paper towels.

6.9 Change gloves.

**IMPORTANT**

The procedure must not exceed 10 minute duration

6.10 Cover the AI gun with the sanitary sheath.

6.11 Insert the AI gun carefully into the vagina with the other hand.

6.12 Guide the AI gun, by trans-rectal manipulation, through the external cervical orifice into the uterus.

6.13 Expose the cytobrush and rotate to collect epithelial cells from uterine horn of choice. Note that this also collects immune cells in the endometrium, but epithelial cells are enriched.

6.14 Retract the cytobrush back to the AI sheath and remove the gun.

6.15 Retrieve the cytobrush from the AI gun and place the tip into a 2 mL cryo-tube containing 1 mL of trizol or other sample collection buffer, to release cells. Freeze the tube in liquid nitrogen for further storage at –80°C.

6.16 Cells may also be centrifuged if a pellet is to be frozen.

6.17 Untie the animal from restraint and return to the stall.

7. **REFERENCES**


### Document Status and Revision History

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