الاخصاب المختبري في حيوانات المزرعة، الابقار مثالا

In Vitro ? in Farm Animals, Bovine as an Example

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Introduction

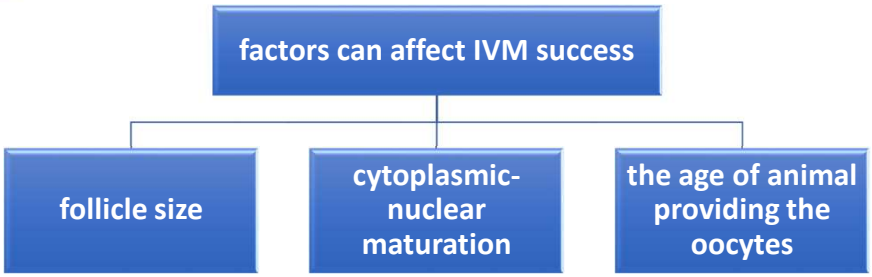
Challenges with *in vitro* production of embryos

- There is a big mistake to name the whole process of *in vitro* as IVF, but the right name is *in vitro* production of embryos (IVP).

- IVP consists of three steps:
  - In vitro maturation (IVM)
  - In vitro fertilization (IVF)
  - In vitro culture (IVC).

- Each step in IVP procedure has problems leading to a decrease of IVP efficiency.

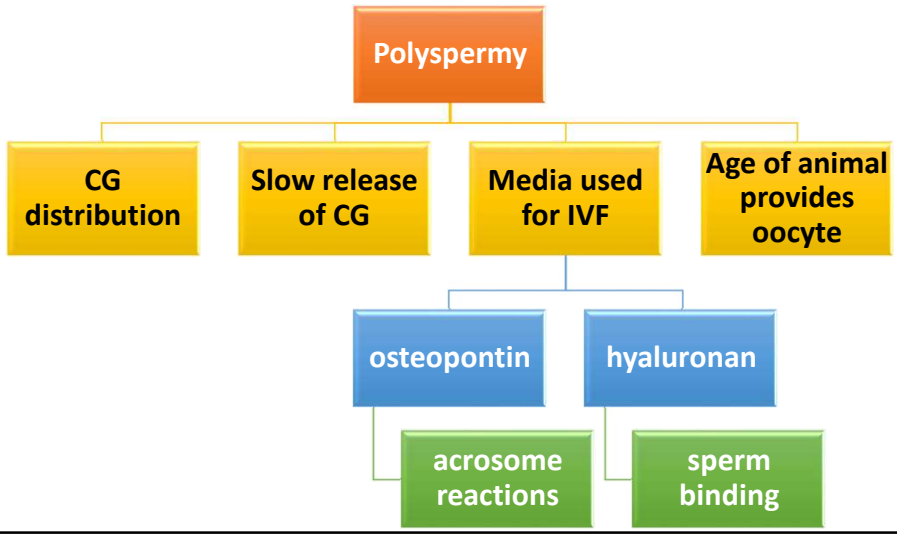
1. Challenges with *in vitro* maturation of oocytes



***In vitro* maturation is the main key to the IVP process's success.**

2. Challenges with *in vitro* fertilization *(IVF)*

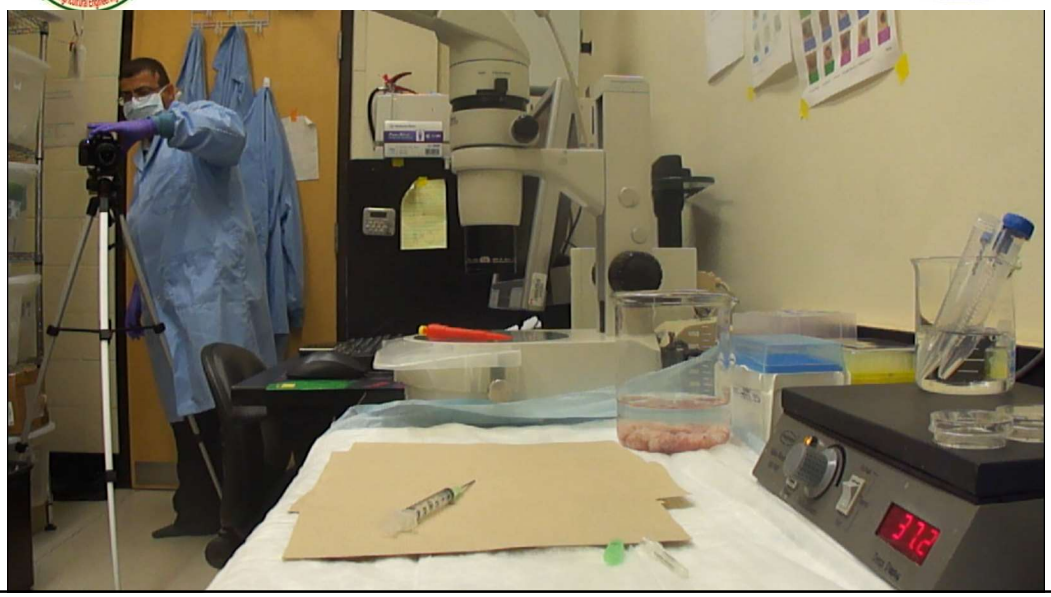
- The main problem affecting IVF is polyspermy



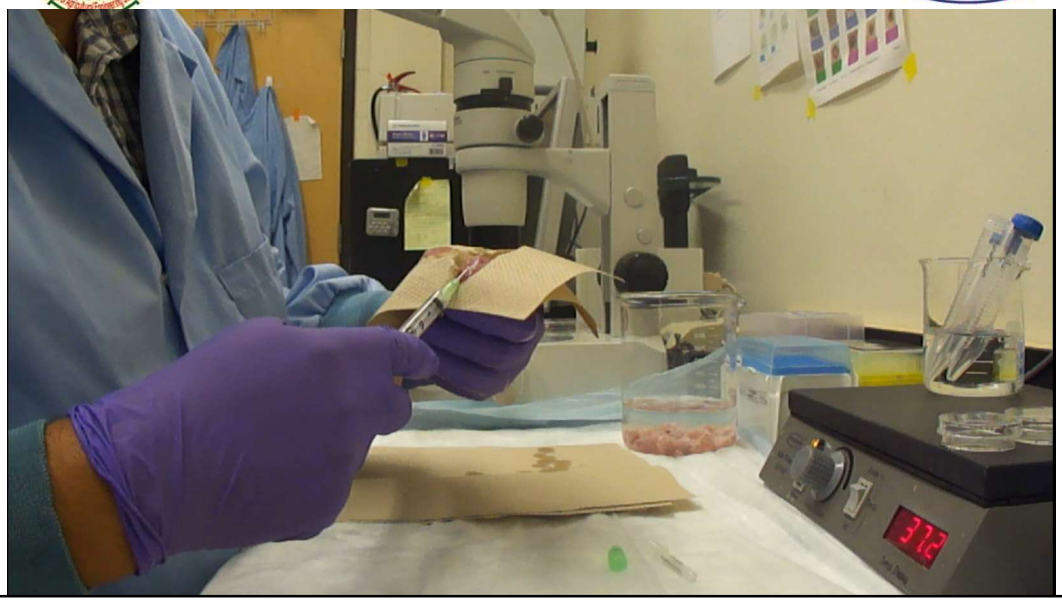
3. Challenges with *in vitro* culture (IVC)

- medium
  - high-energy concentrations
    - Adding some substances
      - melatonin
        - prevent Reactive Oxygen Species (ROS)
    - NCSU-23

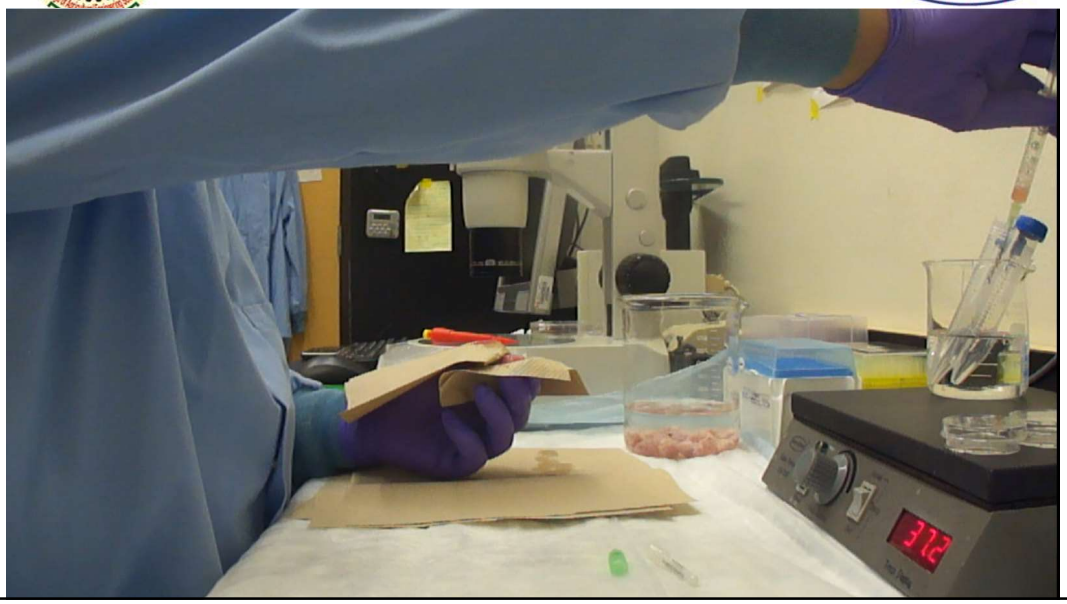
The lab



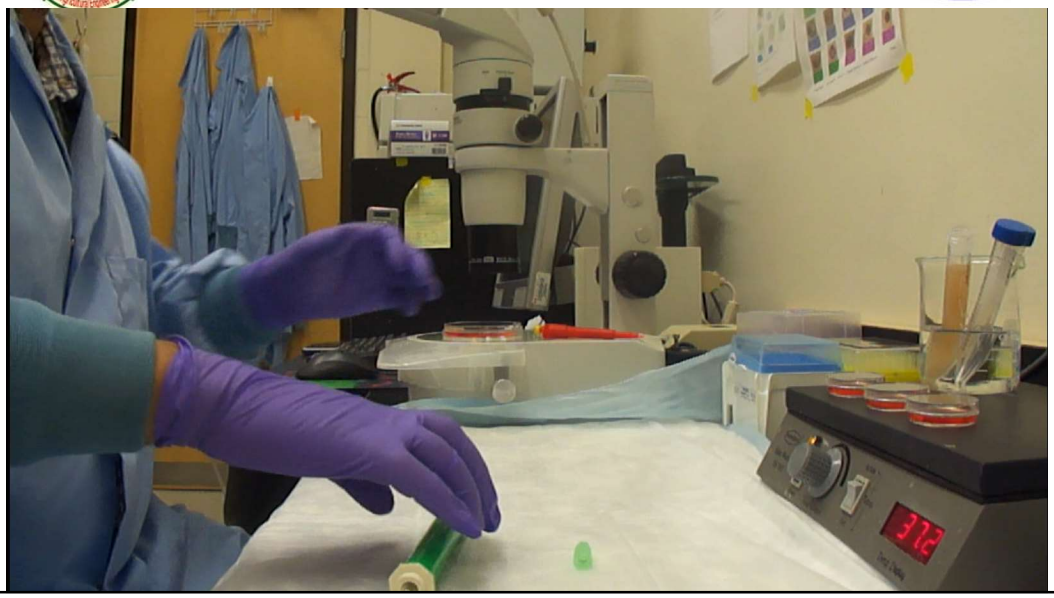
Dry the ovaries and get the COCs



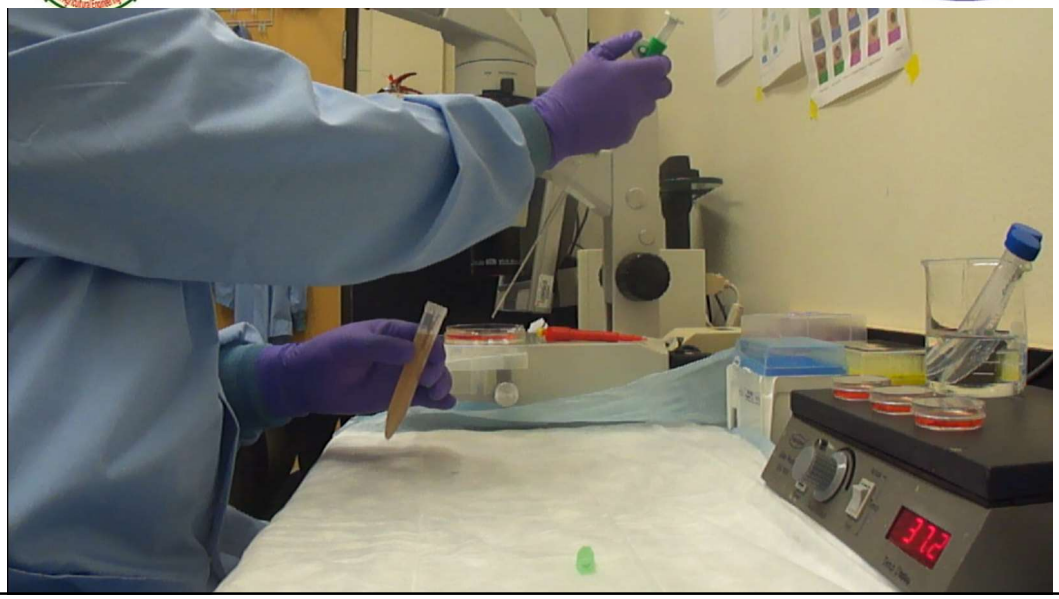
COCs in the water bath



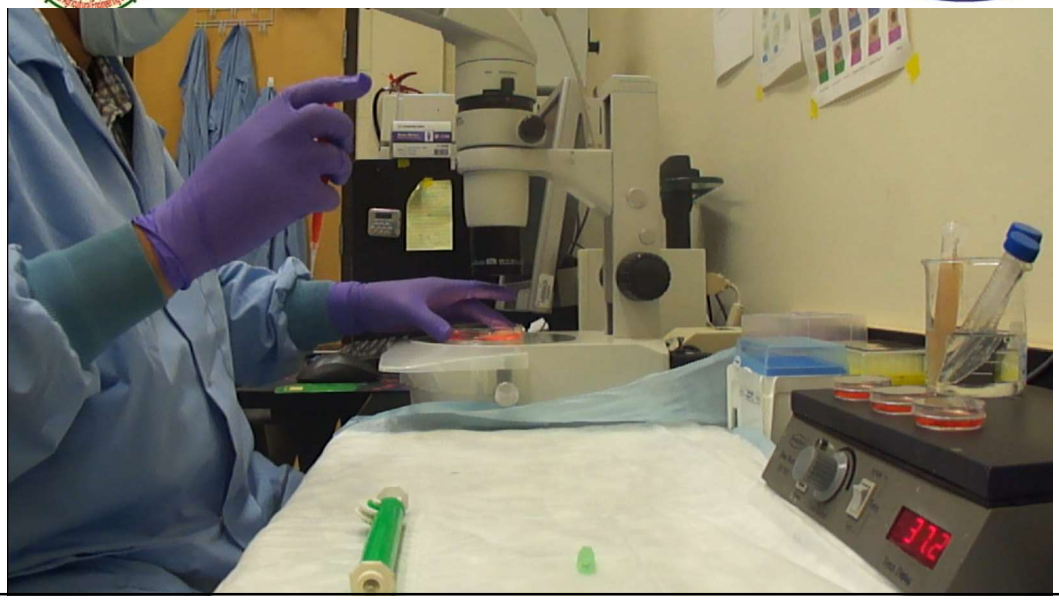
Using a pipet to transfer the COCs



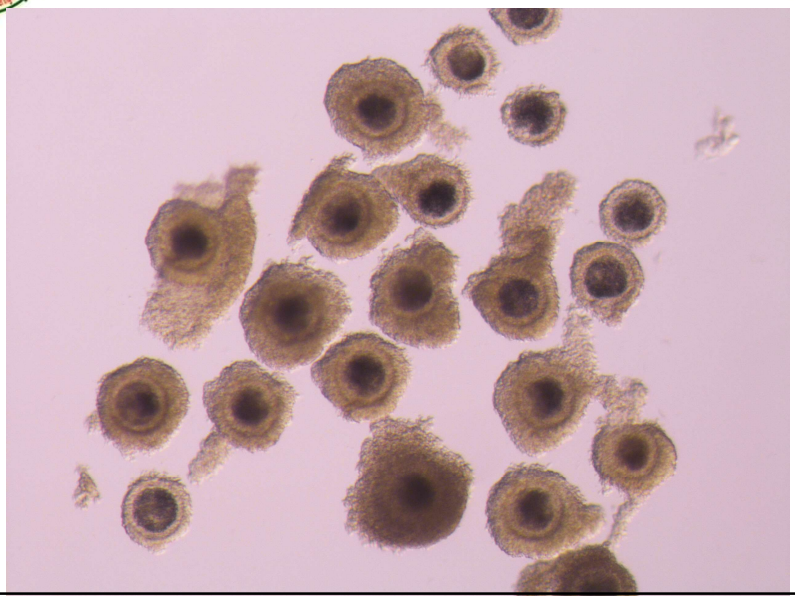
Transfer the COCs from the tube to the washing media



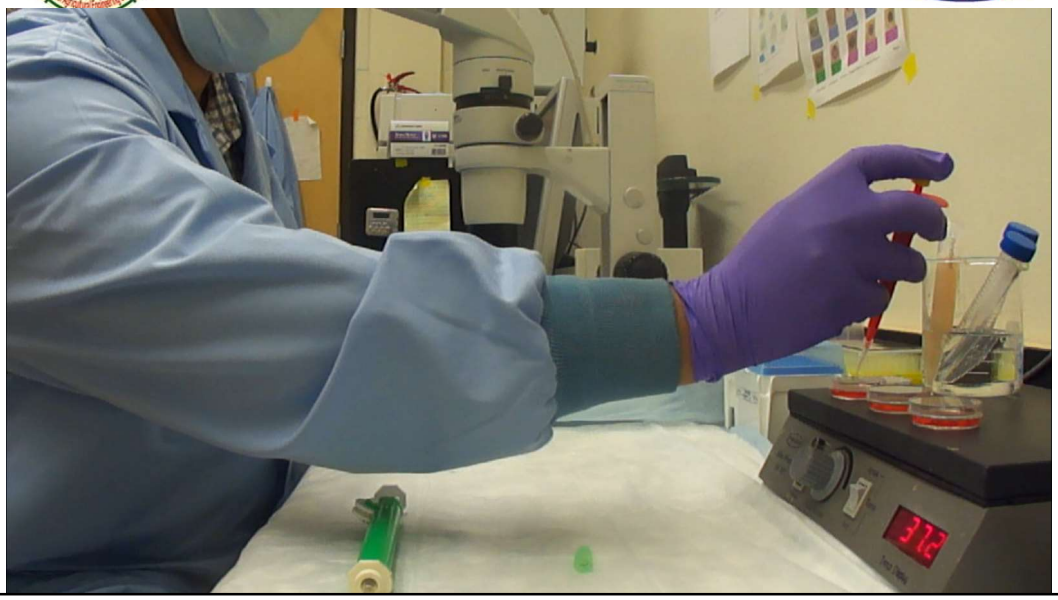
Mixing the COCs and scan them



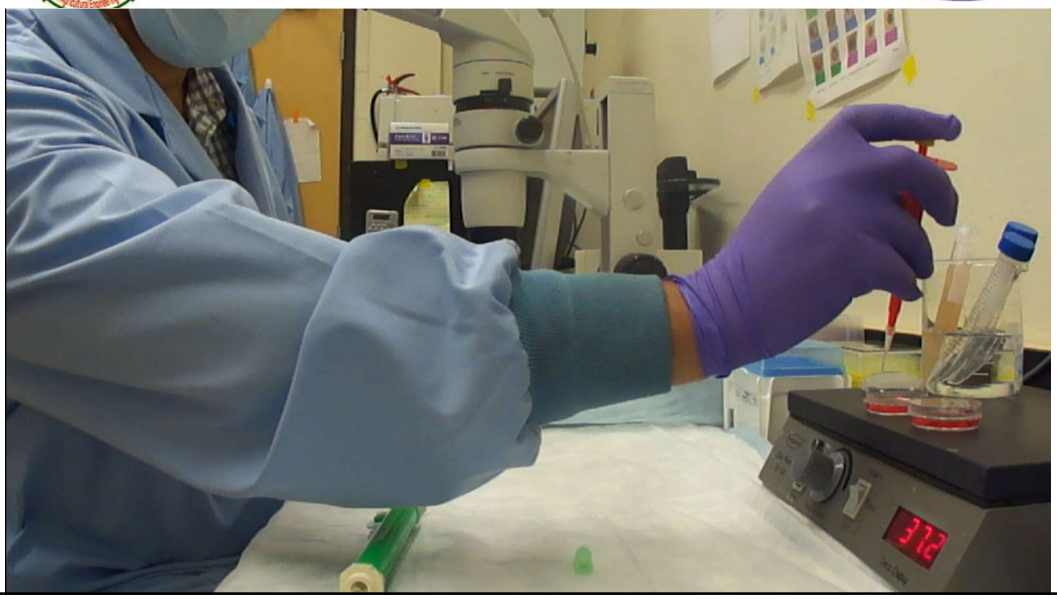
Pick G1 or G2 COCs



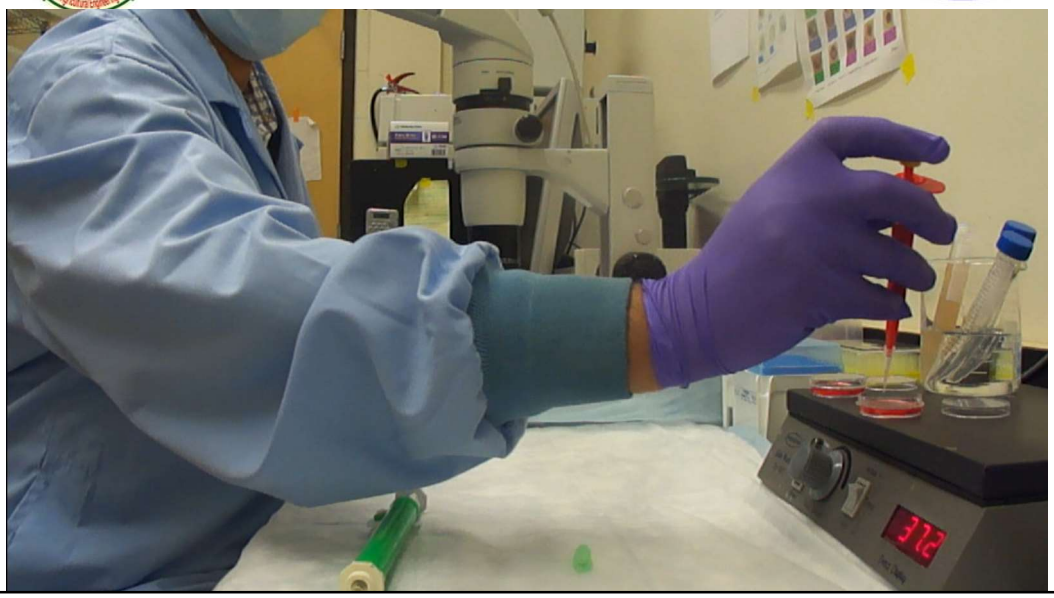
Transfer the COCs to the 1st washing dish



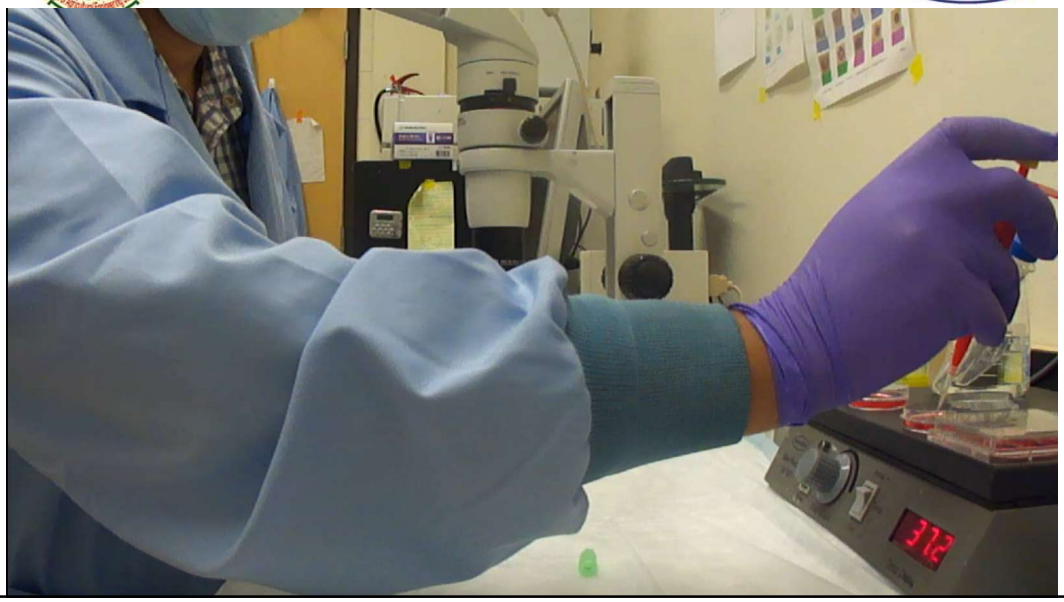
Transfer the COCs to the 2nd washing dish



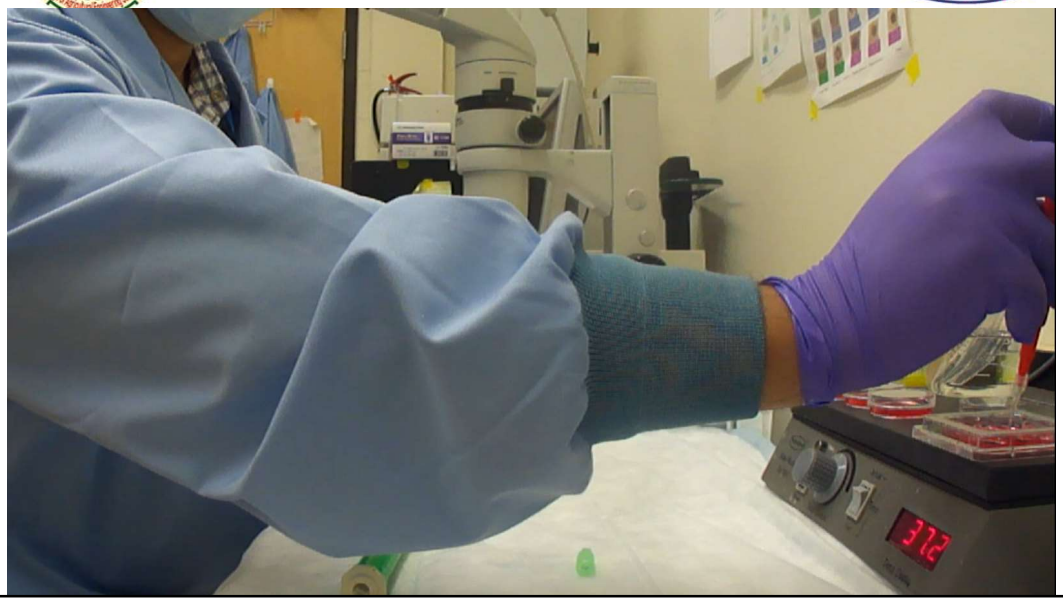
Transfer the COCs to the 3rd washing dish



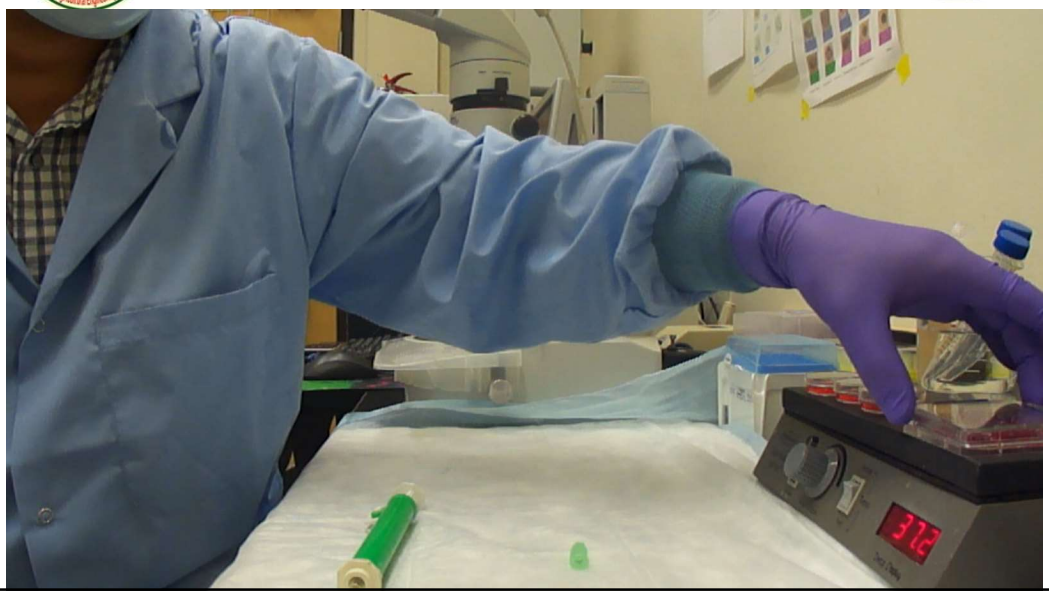
Transfer the COCs to the maturation dish



Transfer the COCs to the 4-well dish



Incubate the 4-well dish in the incubater



Expanded COCs after incubation

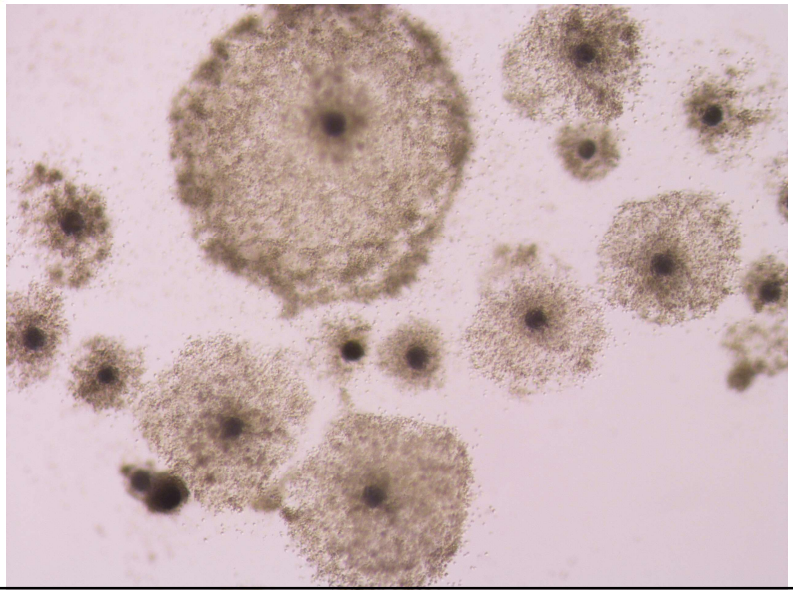


Table 1: Many of the maturation media

	Ringer	PBS	Tyrode	Earle	Hank's	D-Hank's	Dulbecco TCM 199
NaCl	9.00	8.00	8.00	6.80	8.00	8.00	8.00
KCl	0.42	0.20	0.20	0.40	0.40	0.40	0.20
$CaCl_2$	0.25	-	0.20	0.20	0.14	-	0.10
$MgCl_26H_2O$	-	-	0.10	-	-	-	0.10
$MgSO_47H_2O$	-	-	-	0.20	0.20	-	-
$Na_2HPO_4H_2O$	-	1.56	-	-	0.06	0.06	-
$NaH_2PO_42H_2O$	-	-	0.05	0.14	-	-	1.42
KH_2PO_4	-	0.20	-	-	0.06	0.06	0.20
$NaHCO_3$	-	-	1.00	2.20	0.35	0.35	-
Glucose	-	-	1.00	1.00	1.00	-	-
Phenol red	-	-	-	0.02	0.02	0.02	0.02

Yang and Xiong(2012)

Table 2: Culture conditions used for in vitro maturation of bovine oocytes.

Media type	Hormone	Additional Factors	Protein source	Temperature	Atmospherics	IVM time
TCM199	1 µg/mL FSH 12 IU/mL LH 0.1 IU/mL hCG 1 µg/mL 17β-Estradiol	10 ng/mL EGF	10% FBS 0.2-7 mg/mL BSA	38-39 C	5% CO2 in air	22–24 h

Bahrami and Cottee, 2022

Table 3: Effects of two maturation media on in vitro development of bovine in vitro maturation (IVM)-in vitro fertilization oocytes

IVM medium*	No. of oocytes examined (replicates)	Numbers (%) of oocytes developed to					
		≥2 cells	≥8 cells	Morula	Blastocyst at		
					Day 7	Day 8	Day 9
mTCM199	133 (7)	108 (80.3 ±8.4)	88 (64.9± 17. 3)	66 (49. 0± 13.5)	56 (40.8 ± 13.5)	58 (42.4± 14.6)	59 (43.3± 15.3)
mTCM199+ FBS fraction	157 (7)	139 (87.7 ± 6.9)	119 (76.6± 7. 6)	95 (61.4± 9. 0)	69 (46.0± 10.1)	77 (51. 2± 9.9)	81 (53.4 ± 9. 5)

* Modified TCM199 (mTCM199): 10 mmol/L HEPES-buffered TCM199

Note: Values are means ± standard deviations.

Momozawa, 2020

The end

If you are interested in the next steps, I will do other workshops about IVF and IVC.

Thanks