



ISOLATION OF THECA CELLS FROM GOAT OVARIAN FOLLICLES AND EXPRESSION OF *CYP 19 GENE* IN THE ISOLATED CELLS

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INTRODUCTION

- Ovary - responsible for formation and maturation of female gametes and steroidogenesis
- Theca cell (TC) isolation is quite difficult
- To compare the efficacy two methods for TCs isolation
- To assess the isolated cells by analyzing expression of marker genes



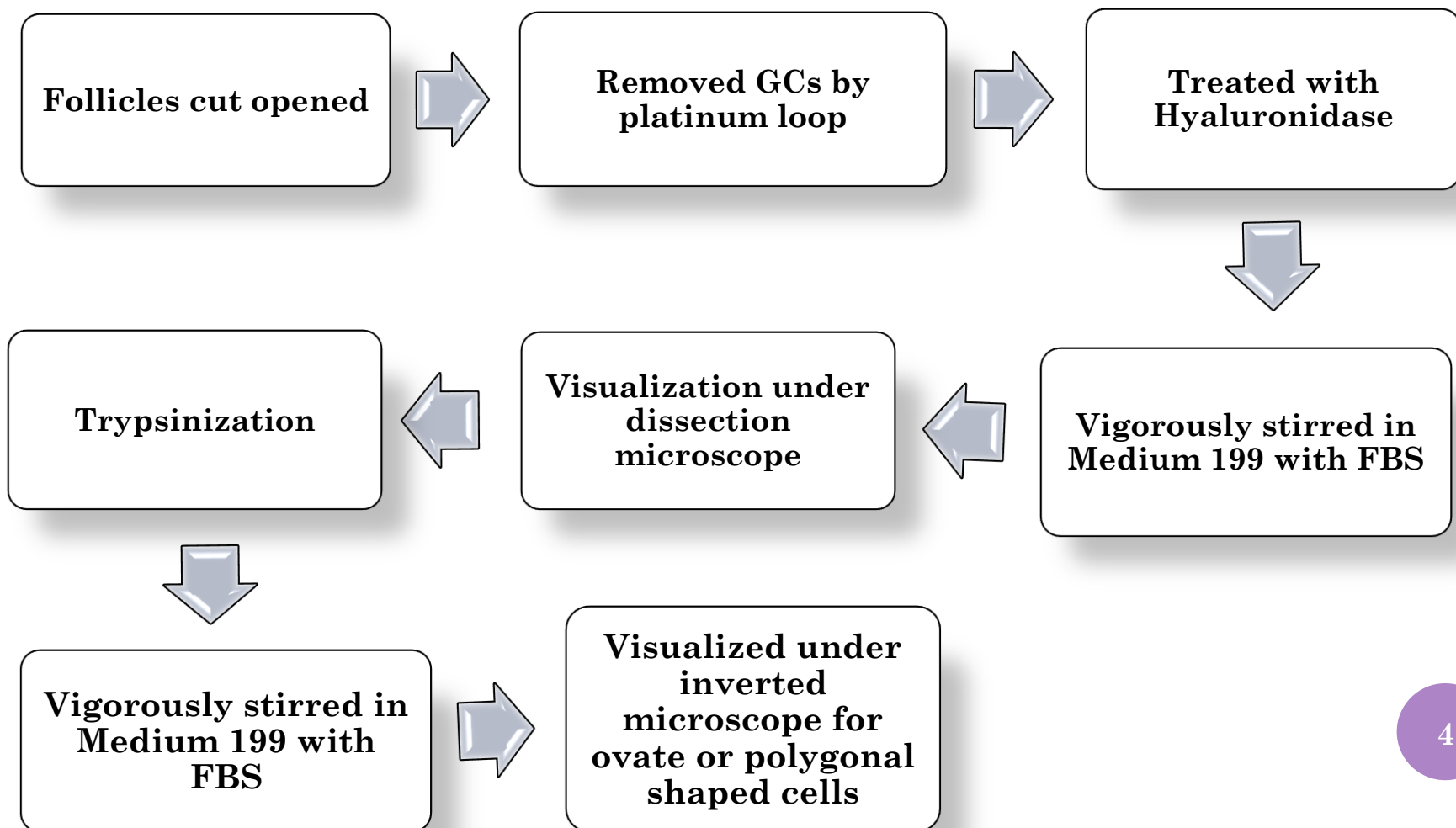
OBJECTIVES

- Isolation of theca cells from goat ovarian follicles by two different protocols
- Expression of *CYP 19* gene in isolated cells to ascertain the best method for isolation of theca cells.



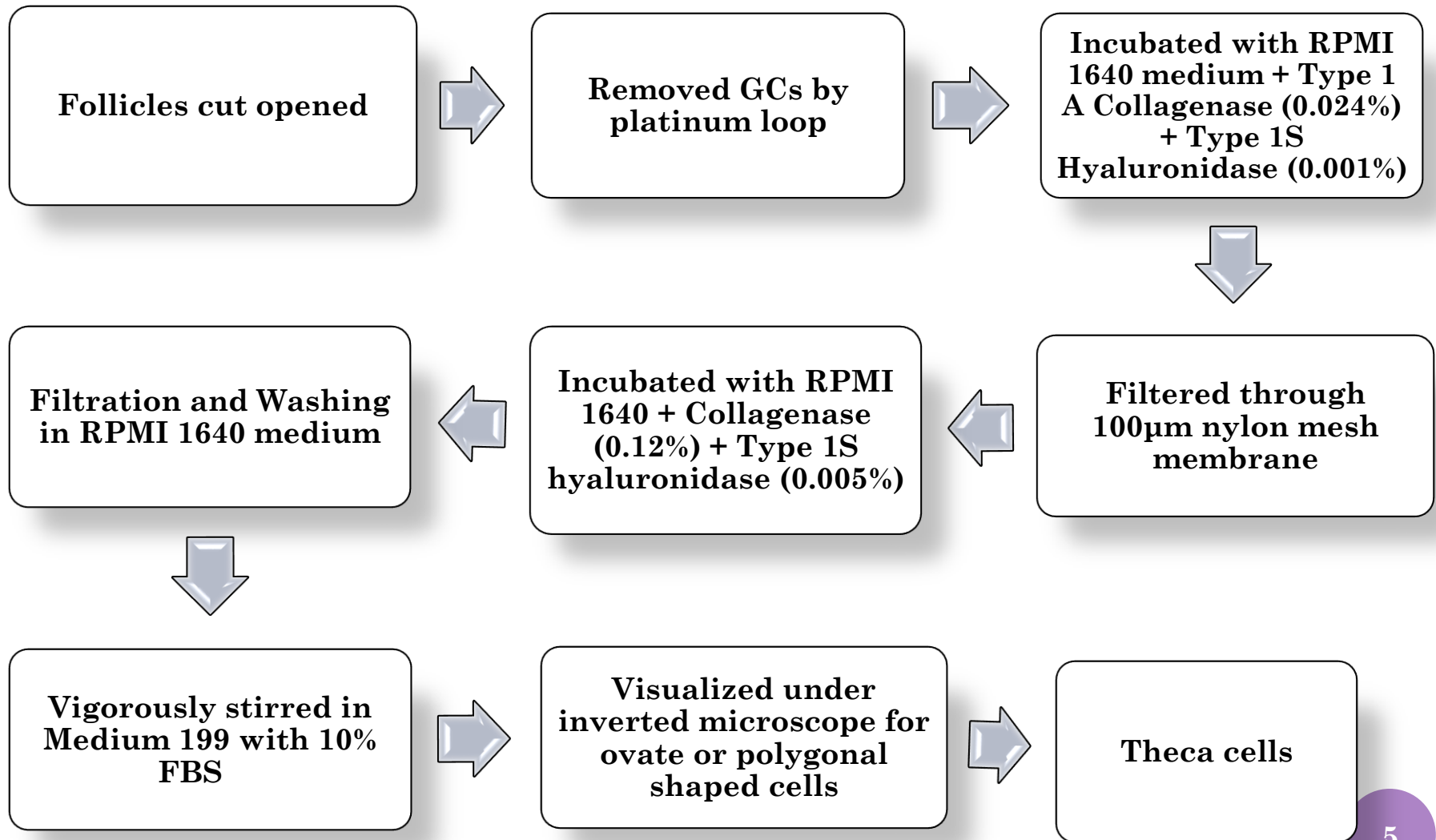
MATERIALS AND METHODS

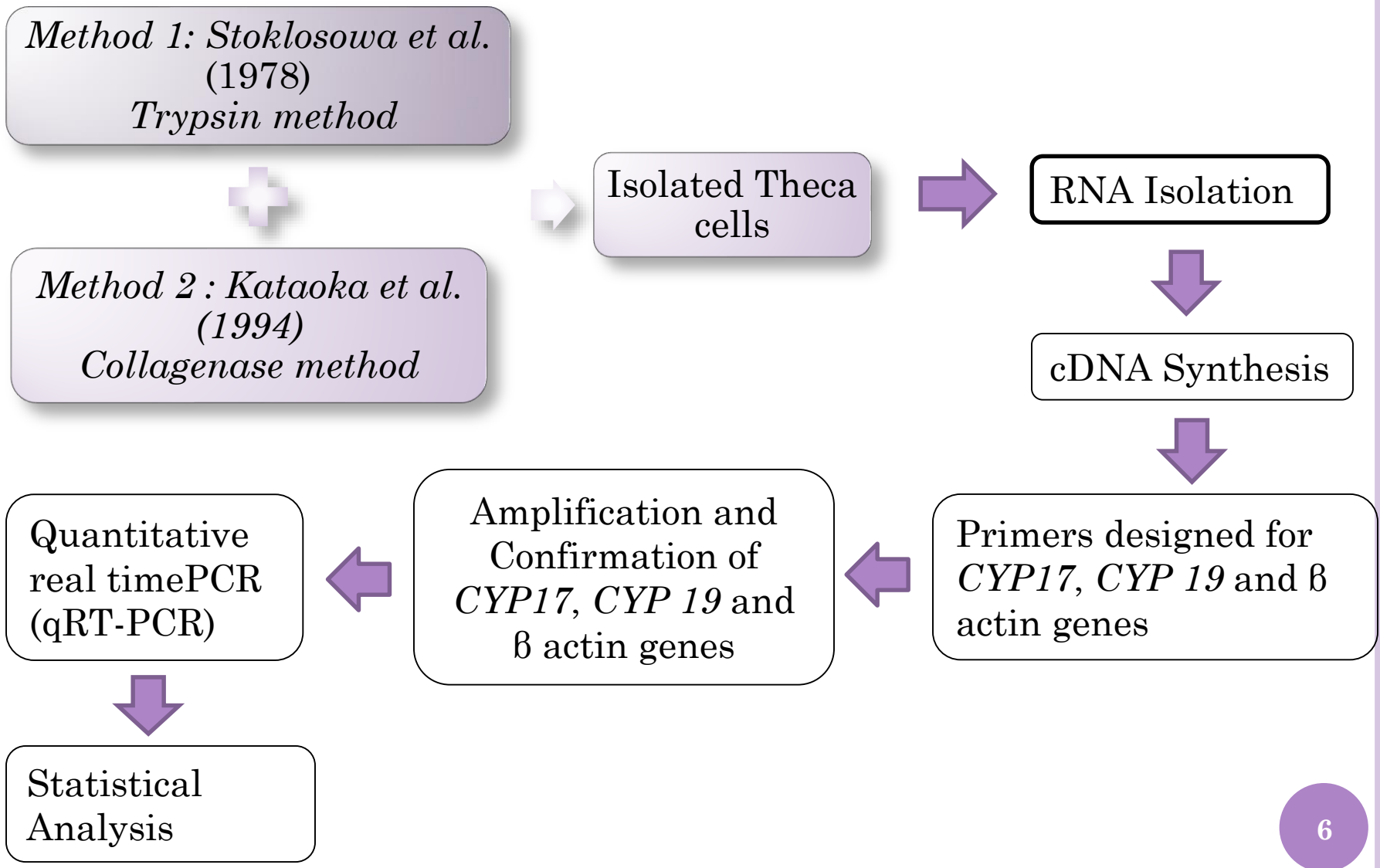
Method 1: Stoklosowa et al. (1978) - Trypsin method





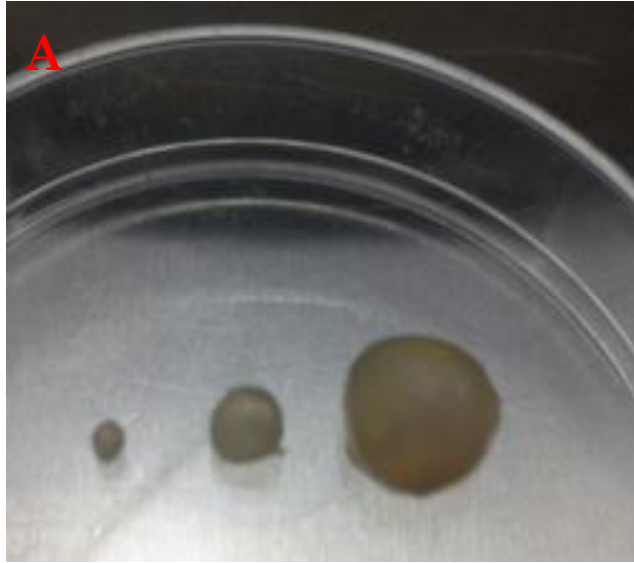
Method 2: Kataoka et al. (1994) - Collagenase method



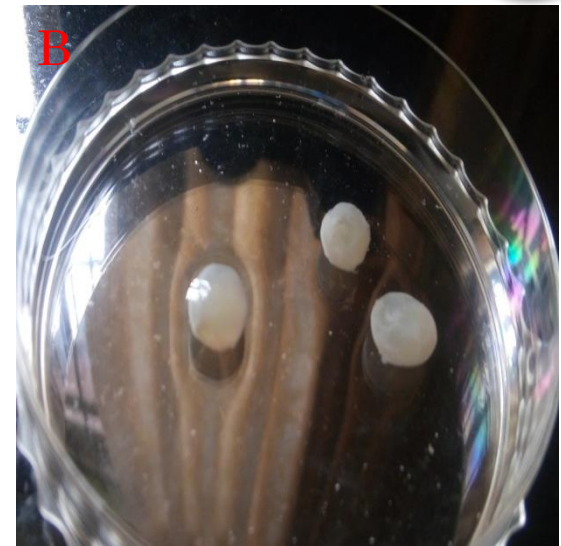




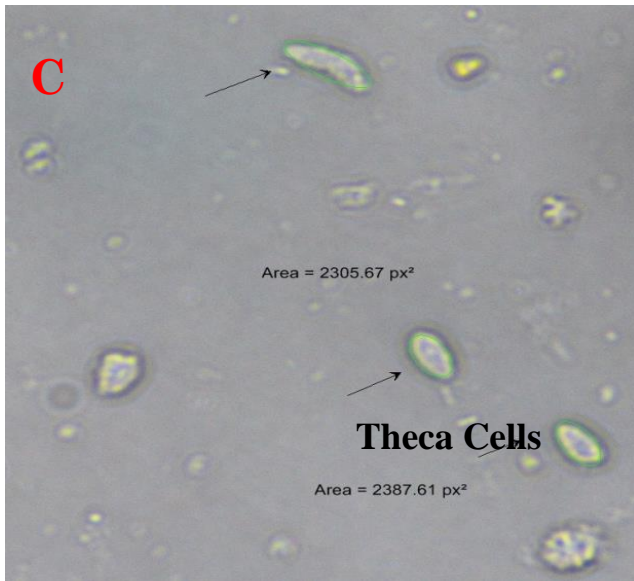
RESULTS



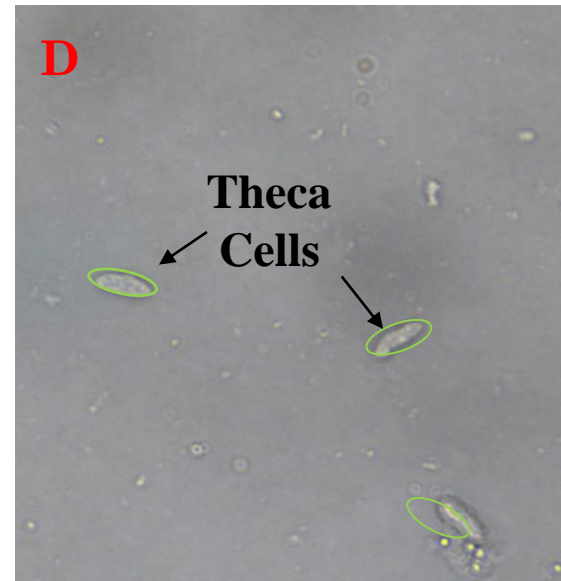
(A) Isolated follicles
(B) 3-6mm diameter of follicles

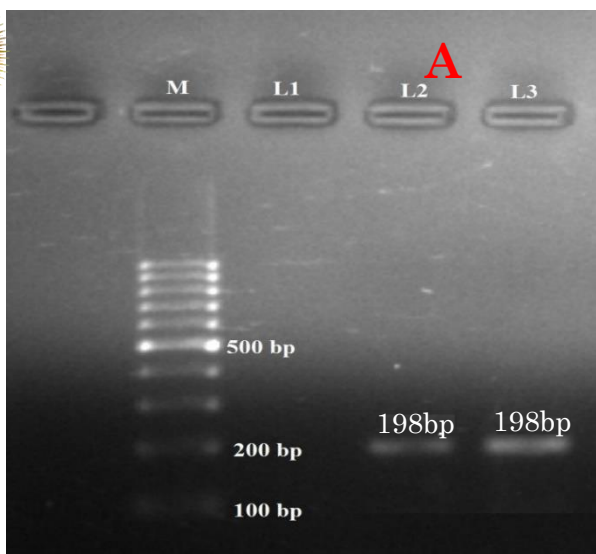


(C) Microscopic Image of Theca Cells (Trypsin) under Inverted Microscope

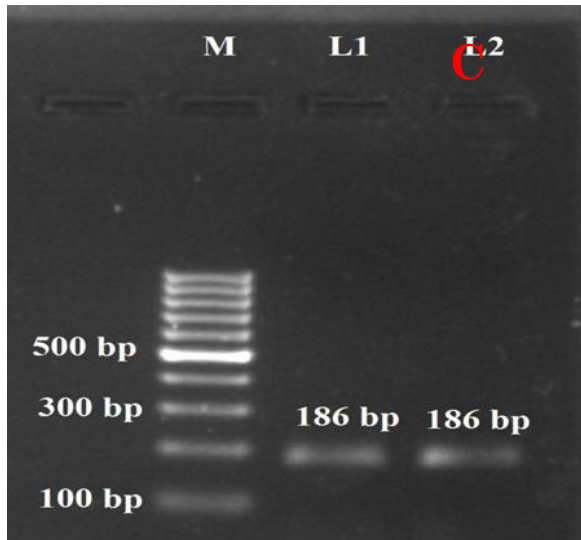


(D) Microscopical Image of Theca Cells (Collagenase) under Inverted Microscope

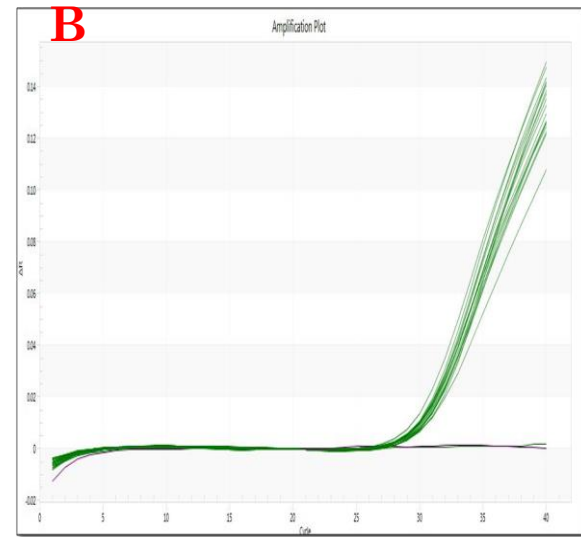




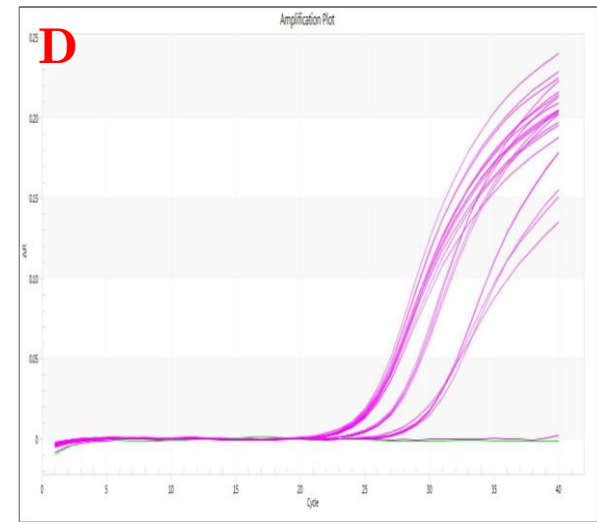
Agarose Gel Electrophoresis of PCR Products of *CYP19* by Trypsin (L2) and Collagenase (L3) along with 100bp DNA ladder (M)



Agarose Gel Electrophoresis of PCR products of *CYP 17* by Trypsin (L1) and Collagenase (L2) along with 100bp DNA ladder (M)



CYP 19 qRT-PCR Amplification Plot



CYP 17 qRT-PCR Amplification Plot

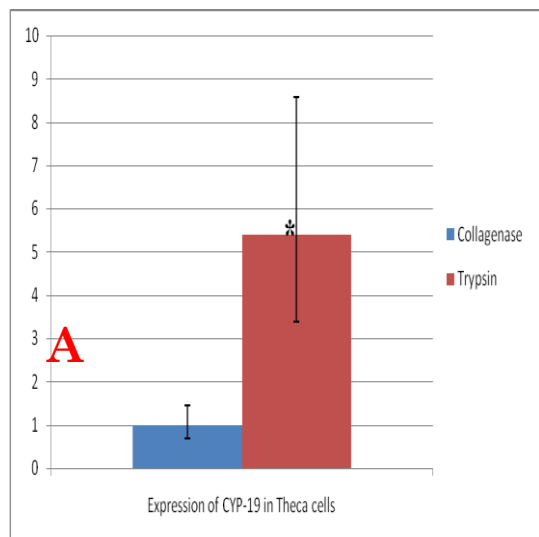


CYP 19 gene expression in between methods (Trypsin and Collagenase)

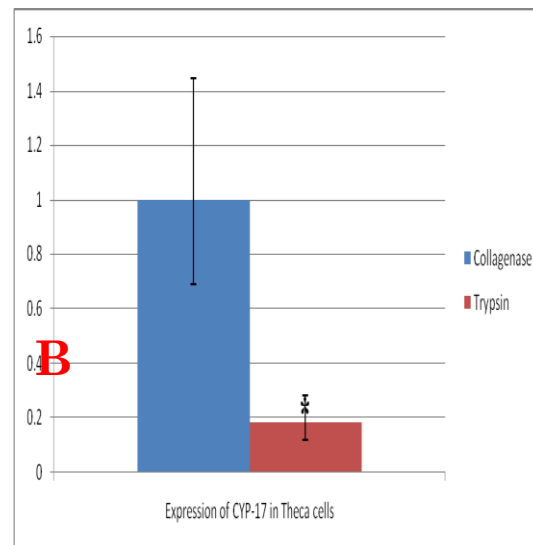
Method	Mean C _T ± S.E n=6		ΔC _T ± S.E	ΔΔC _T ± SE	Fold change from control (2 ^{-ΔΔC_T})	p-Value
	CYP 19	B-actin				
Trypsin	26.03 ± 0.65	18.44 ± 0.16	7.59 ± 0.67	-2.41 ± 0.67	5.38* (3.37-8.58)	0.028
Collagenase	27.37 ± 0.11	17.36 ± 0.52	10.0 ± 0.53	0.00 ± 0.53	1 (0.68-1.44)	

CYP 17 gene expression between methods (Trypsin and Collagenase)

Method	Mean CT ± S.E		ΔC _T ± S.E	ΔΔC _T ± S.E	Fold change from control (2 ^{-ΔΔCT})	P-Value
	CYP 17	B-actin				
Trypsin	24.65 ± 0.62	18.41 ± 0.14	6.23 ± 0.64	2.46 ± 0.64	0.18 (0.69-1.44)	0.0169
collagenase	21.12 ± 0.11	17.36 ± 0.52	3.76 ± 0.53	0.00 ± 0.53	1 (1.82-3.10)	



CYP 19 gene expression in between methods (Trypsin and Collagenase)



CYP 17 gene expression in between methods (Trypsin and Collagenase)



CONCLUSION AND APPLICATIONS

- Confirms that collagenase method is the better one for isolation of TCs from goat ovarian follicles.
- Study the functions in follicular development and ovarian steroidogenesis



**Thank you for your
attention**