Isolation and Characterization of (primary) Bovine Luteal Cells

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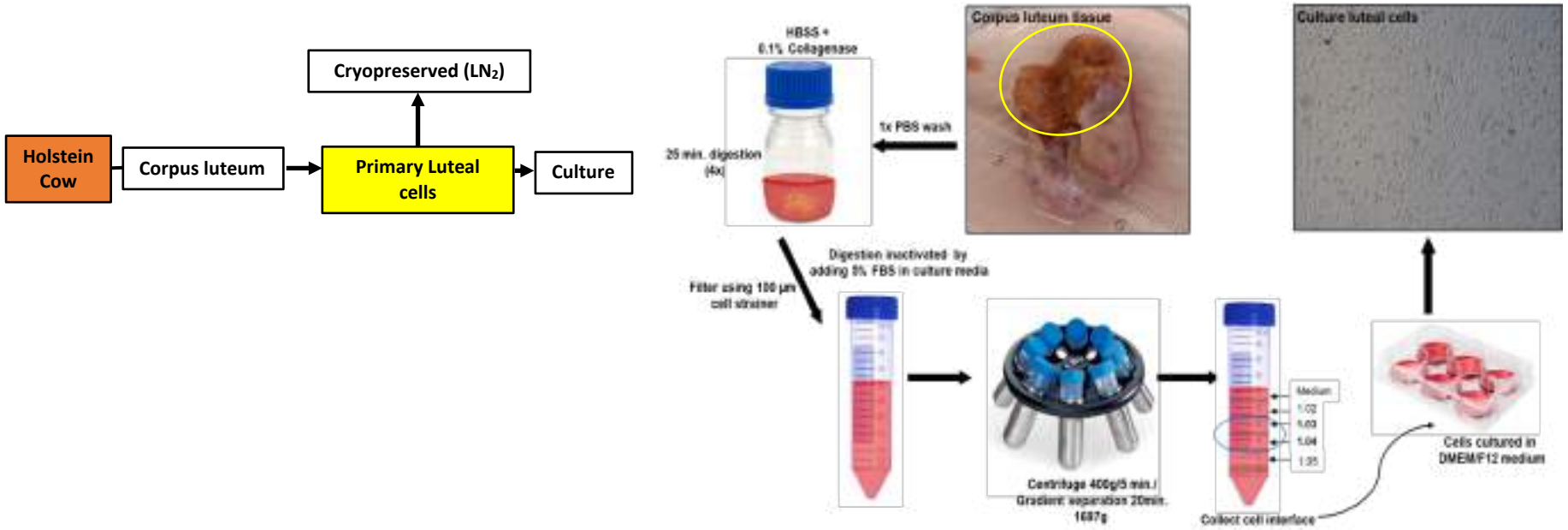
WP1 Isolation and Characterization of (primary) Bovine Luteal Cells

BovReg WP1: Development of Laboratory Tools and Resources
Task 1.1 Generation and Characterization of new bovine cell lines



Luteal cells isolation by Percoll gradient method

Primary luteal cells

Transfected luteal cells

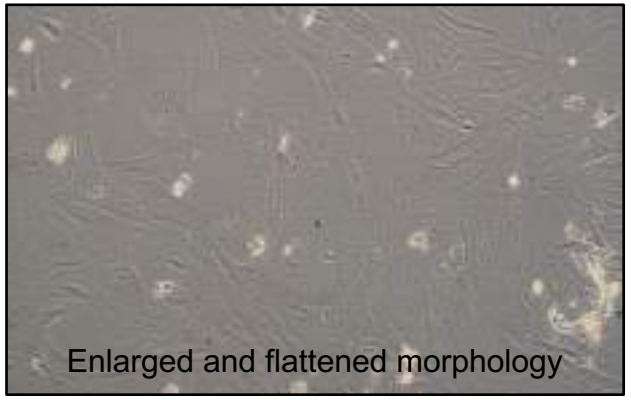
Cultured

Transfection (hTERT gene)

Selection (G418 antibiotic)

Expansion

Transfected Luteal cell lines showed very slow growth post passage 6, with hTERT gene expression atleast until passage 6 but cells failed to proliferate further



TransIT-X2 (mirus bio MIR 6003) transfection reagent
Plasmid: pCIneo-hEST2
Mirus G418 Sulfate Solution 50mg/ml

Non-transfected luteal cells

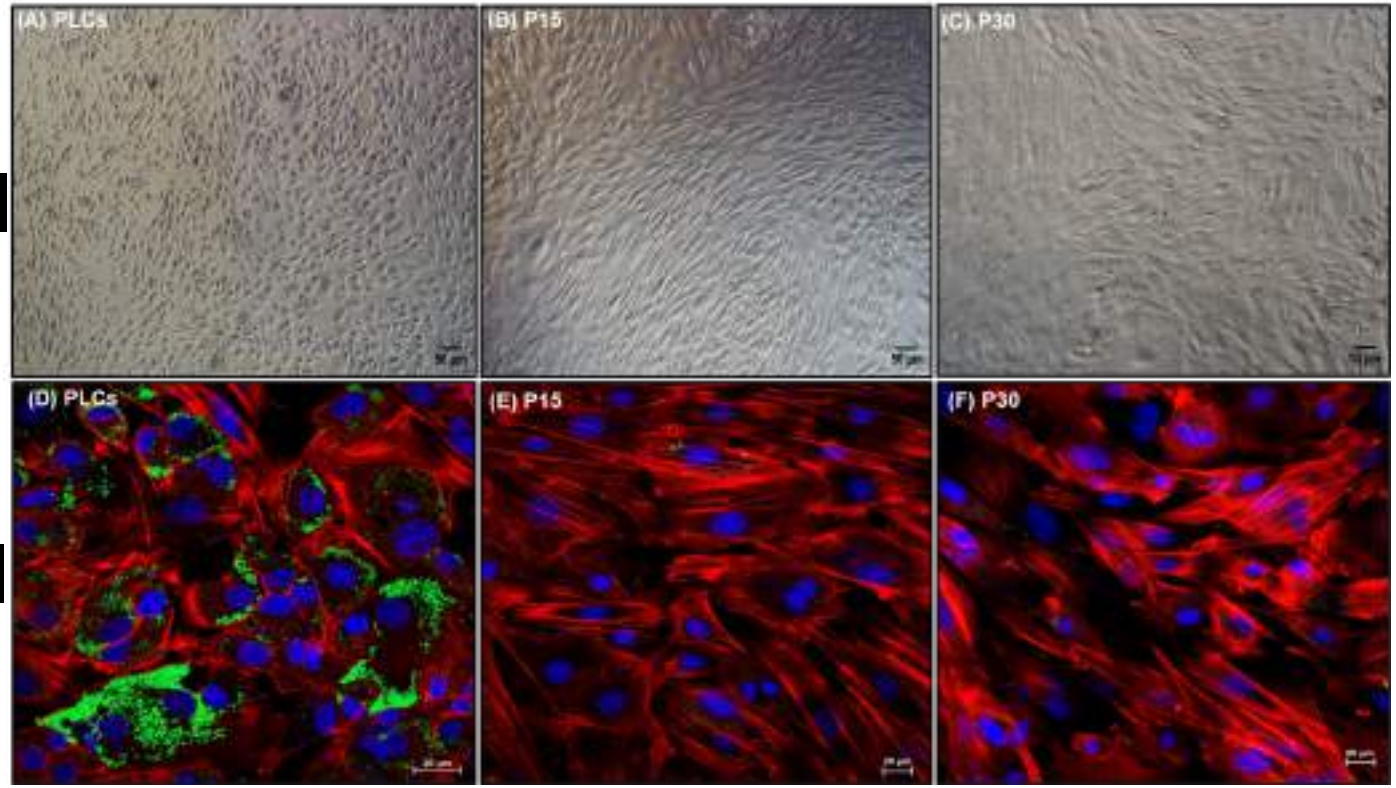
In vitro passaging (30 passages)

Luteal cells were characterized at primary, intermediate passage (P15) and high passage (P30)

Luteal cells with higher passage number exhibit alterations in morphology and functions

Cell morphology

Lipid content



- Primary luteal cells displayed normal morphology compared to enlarged and flattened morphology observed at higher passages (p15 and p30)
- With *in vitro* passaging lipid content is significantly reduced, low lipid content affects the function of **steroidogenesis (progesterone synthesis)** in luteal cells

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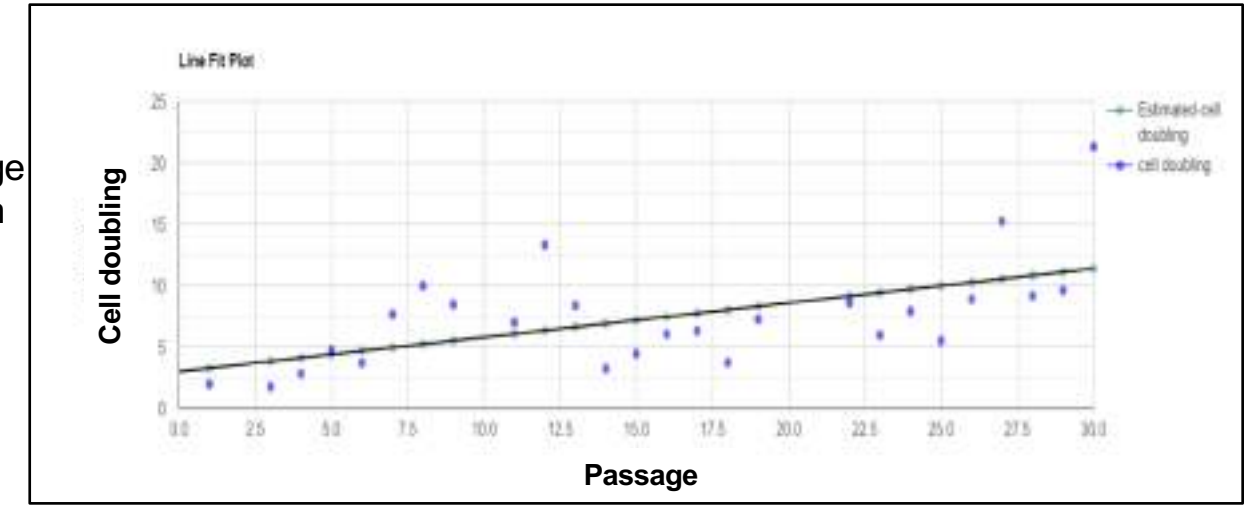
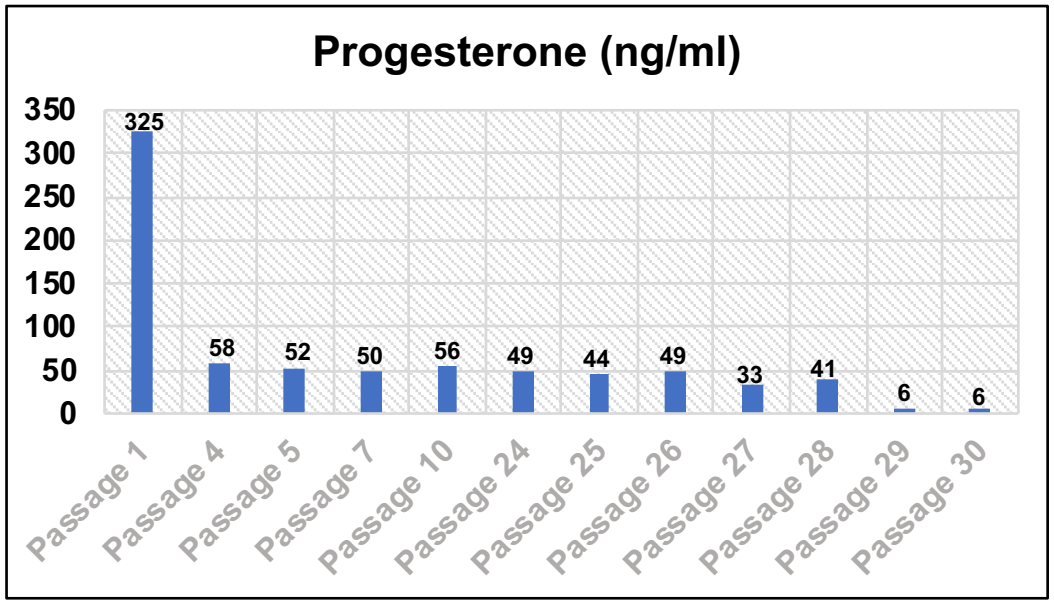
Cell doubling rate is related to passage number and increases significantly with passage number





Synthesis of progesterone (P4) is severely compromised in luteal cells with successive passaging

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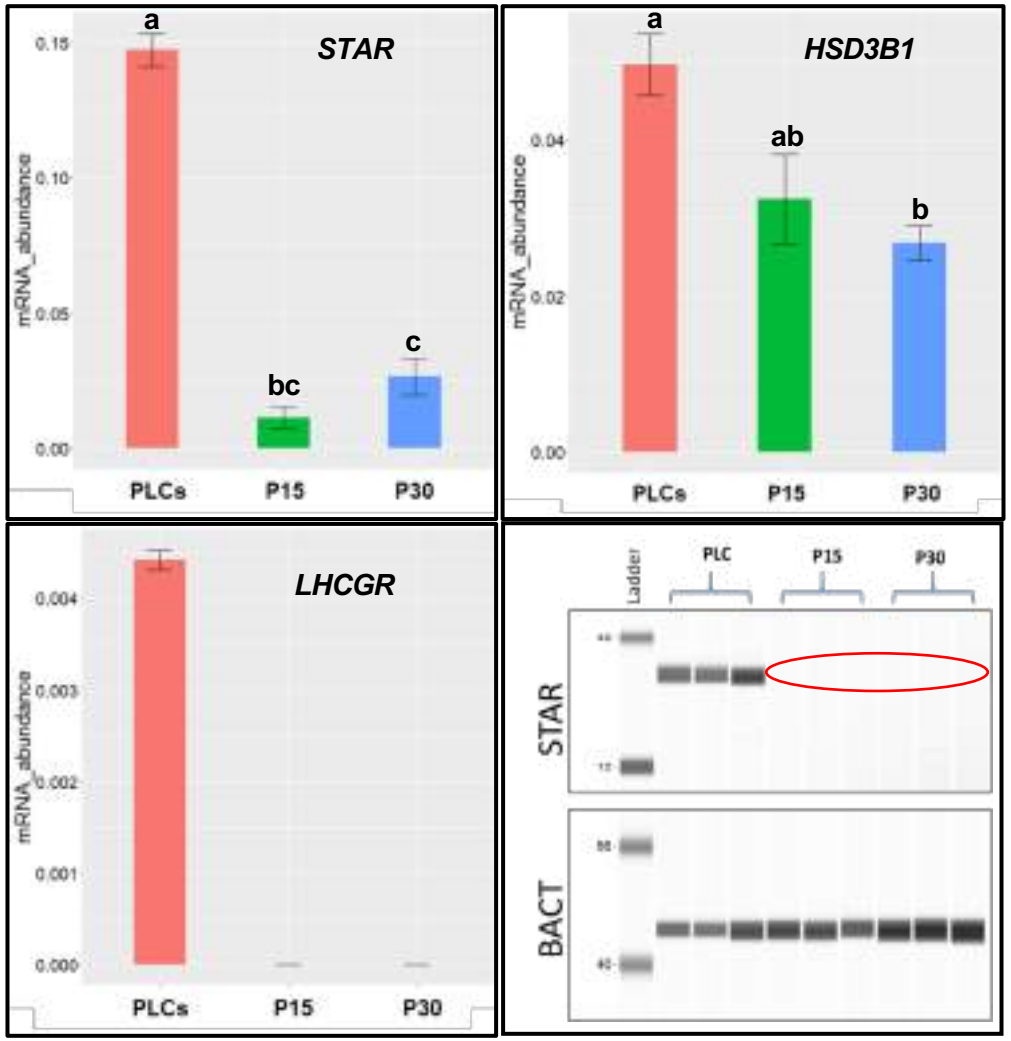
In vitro passaging adversely affects the marker gene expression of luteal cell function

- Synthesis of P4 by luteal cells requires **STAR, LHCGR** and **HSD3B1** as key functional proteins
- With passaging, the relative mRNA expression levels of *STAR*, *HSD3B1* were significantly downregulated
- Higher passaged luteal cell looses STAR and LHCGR protein expression



HSD3B1: hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1
LHCGR: luteinizing hormone/choriogonadotropin receptor
STAR: steroidogenic acute regulatory protein
BACT: β actin

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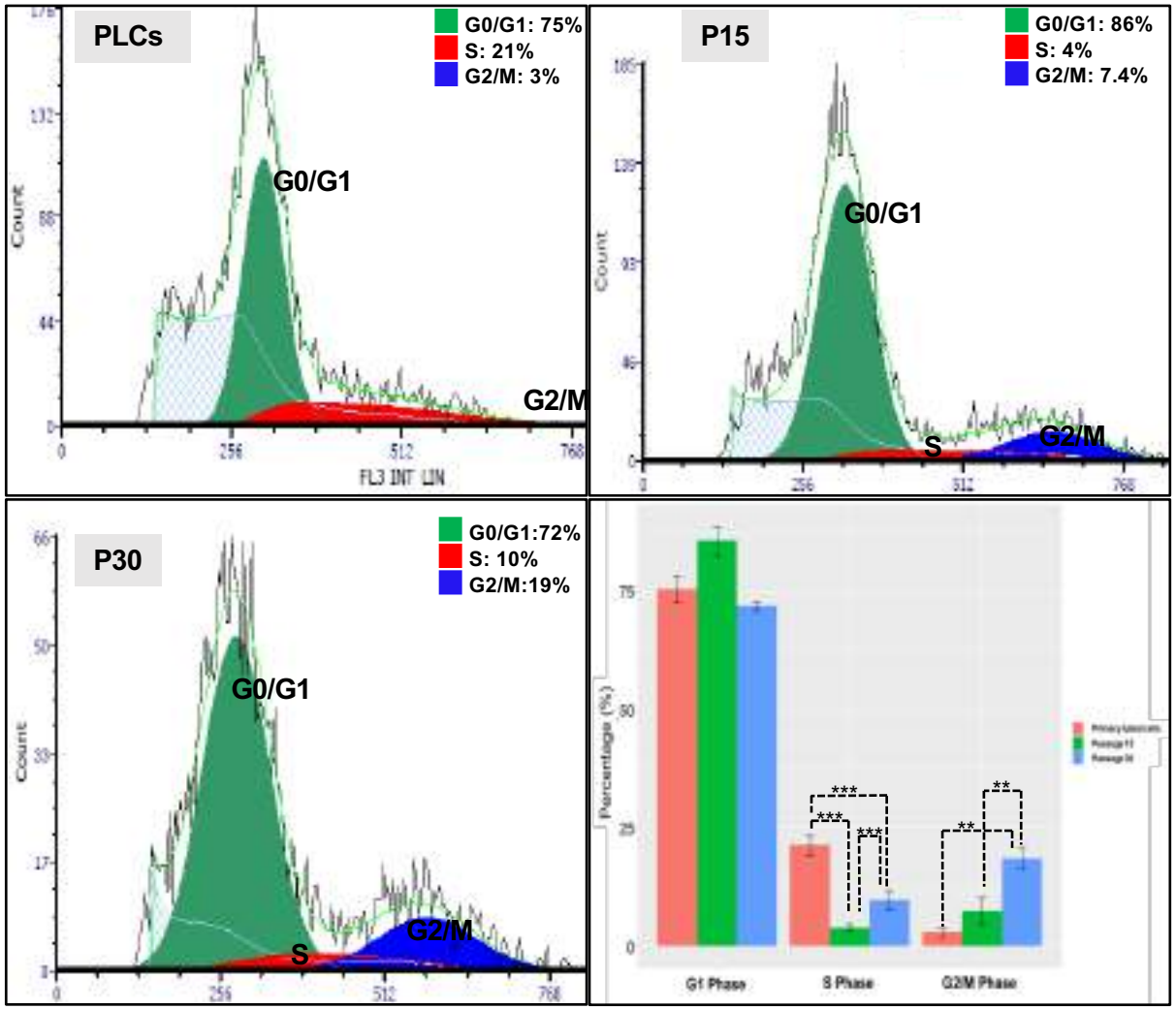
Cell proliferation decreases at high passages



- With passaging, the proportion of luteal cells in S-phase (replicative phase) is significantly reduced
- With passaging, cell proliferation is significantly delayed

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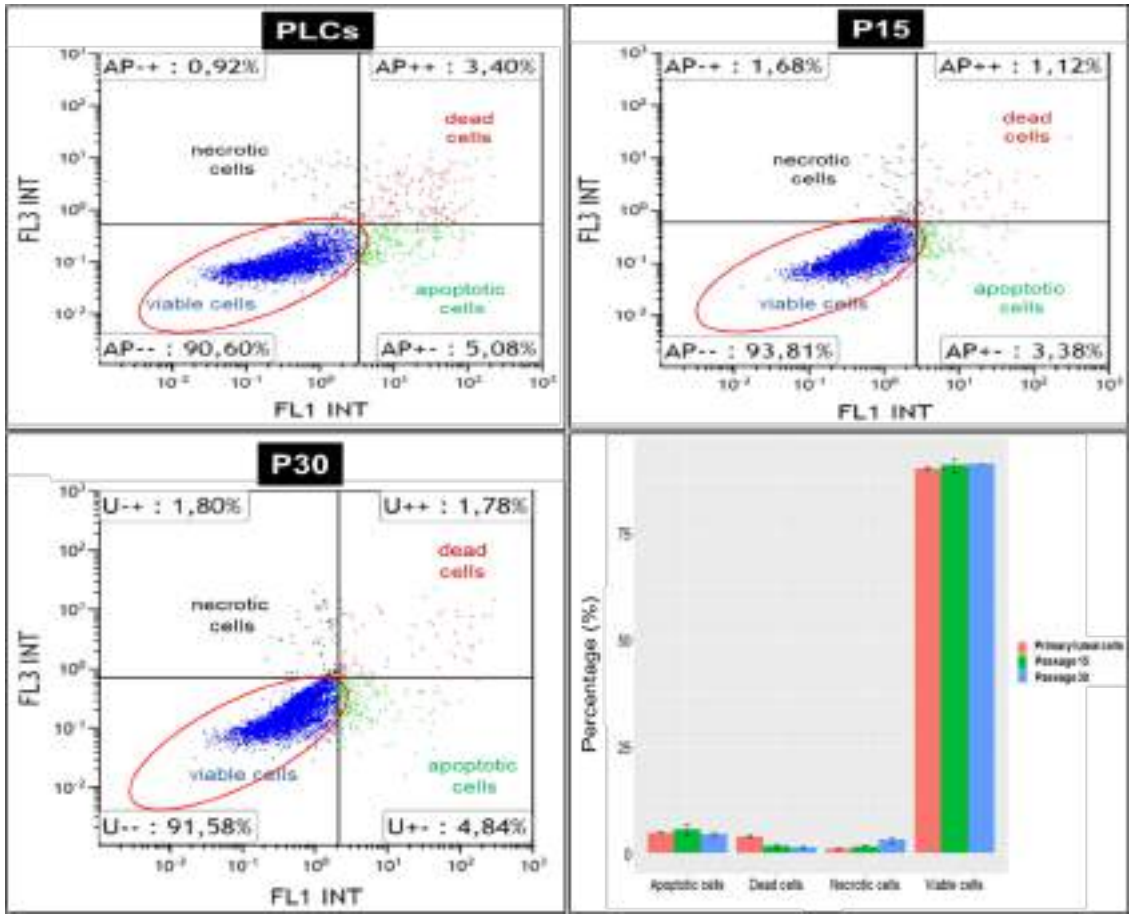
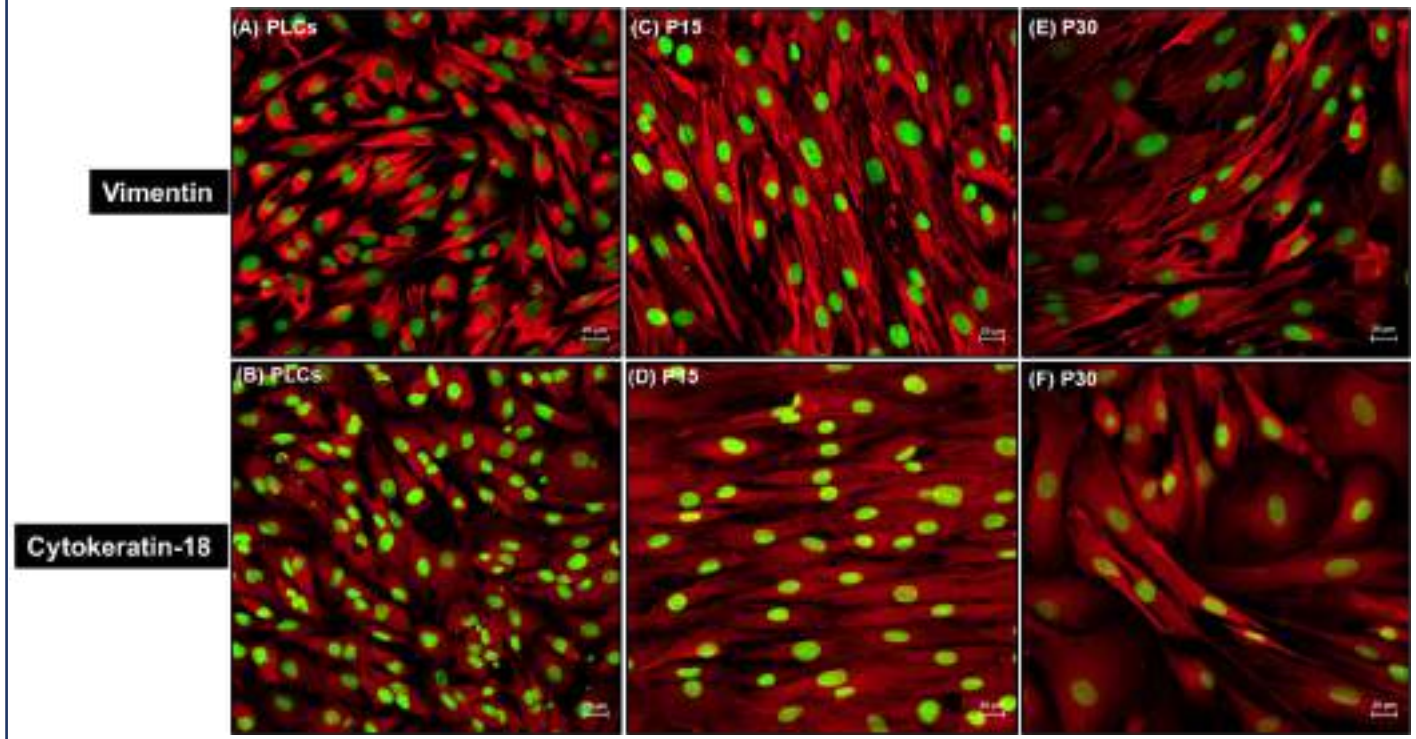
Luteal cell viability is unaffected by passage number

- Approx. 90% viable cells observed in primary, intermediate passage (p15) and late passage (p30) luteal cells
- Results suggests that proteins and pathways involved in cell survival might remain functional in long term passaged luteal cells



Expression of cytoskeleton proteins remain stable from early to late passaged luteal cells



- Cell cytoskeleton marker proteins vimentin and KRT-18 were co-expressed in luteal cells throughout the passaging

- Luteal cells structural integrity remains consistent irrespective of passage number

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Luteal cell transcriptome:

RNA sequencing revealed a total of 13,763 expressed genes in luteal cells

Functional enrichments (Top 500 expressed genes)

Biological Process (Gene Ontology)

GO-term	description	count in network	strength	false discovery rate
GO:2000435	Negative regulation of protein neddylation	3 of 4	1.52	0.0120
GO:1905323	Telomerase holoenzyme complex assembly	3 of 4	1.52	0.0120
GO:0044849	Estrous cycle	3 of 4	1.52	0.0120
GO:0010757	Negative regulation of plasminogen activation	4 of 6	1.47	0.0020
GO:1903377	Negative regulation of oxidative stress induced neuron intri...	3 of 5	1.42	0.0177

(more ...)

Molecular Function (Gene Ontology)

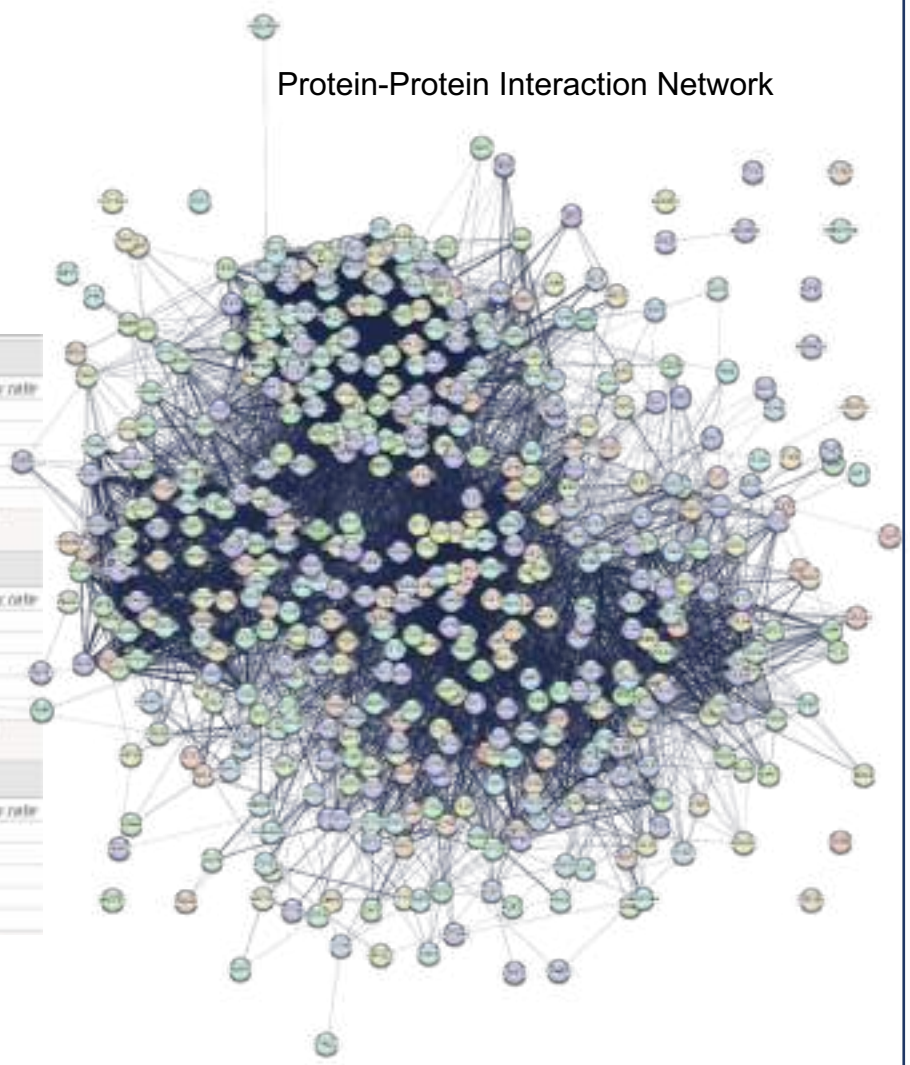
GO-term	description	count in network	strength	false discovery rate
GO:1990948	Ubiquitin ligase inhibitor activity	5 of 9	1.56	0.00023
GO:0043532	Angiostatin binding	3 of 4	1.52	0.0151
GO:0004656	Procollagen-proline 4-dioxygenase activity	3 of 5	1.42	0.0228
GO:0048407	Platelet-derived growth factor binding	5 of 9	1.39	0.00034
GO:0046933	Proton-transporting ATP synthase activity, rotational mecha...	6 of 11	1.38	0.00033

(more ...)

Cellular Component (Gene Ontology)

GO-term	description	count in network	strength	false discovery rate
GO:0034674	Integrin alpha5-beta1 complex	2 of 2	1.64	0.0331
GO:0005584	Collagen type I trimer	2 of 2	1.64	0.0331
GO:0097513	Myosin II filament	3 of 4	1.52	0.0058
GO:0098556	Cytoplasmic side of rough endoplasmic reticulum membrane	4 of 6	1.47	0.00090
GO:0030478	Actin cap	2 of 3	1.47	0.0491

Protein-Protein Interaction Network



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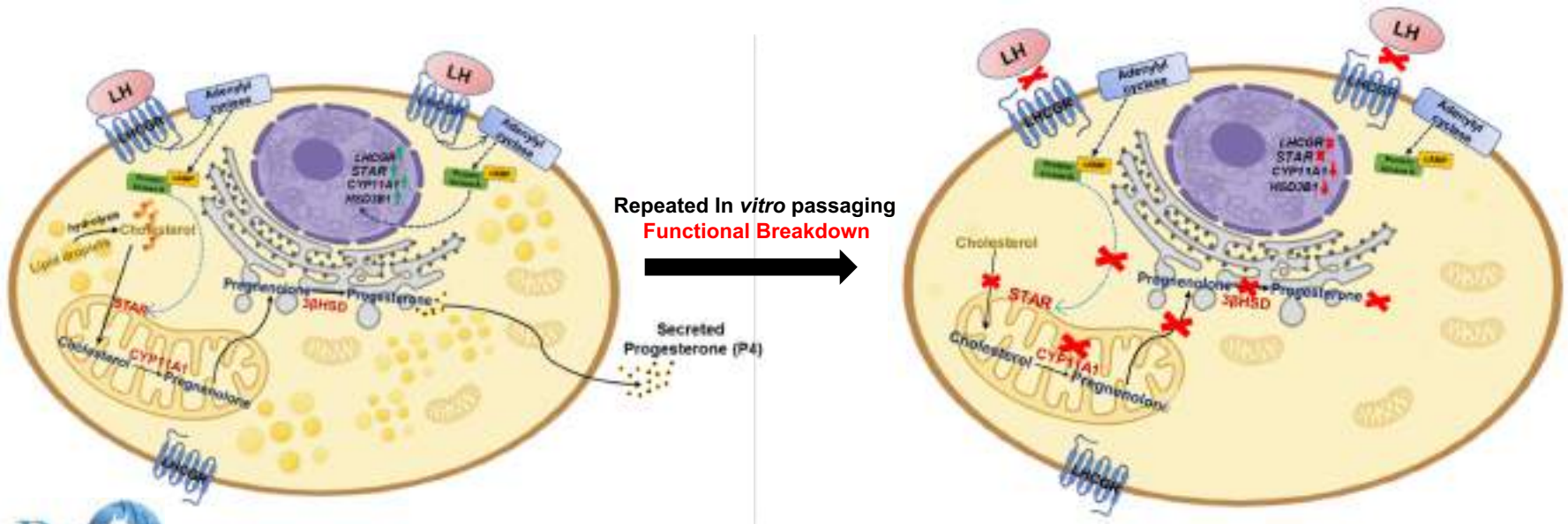
Summary:

- *In vitro* passaging has adverse effect on long-term cultured luteal cells as they loose their signature marker genes (*STAR, LHCGR* and *HSD3B1*) essential for P4 synthesis
- Cell viability and structural integrity remains intact throughout subsequent passaging *in vitro*
- Repeatedly passaged to proliferate, the key functions of specialized luteal cells are either altered or diminished, which can potentially affect the experimental outcomes
- It is highly recommended to define and set the passage number while using cells in long-term culture experiments (especially in cell lines)



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Thank you for your attention

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