Carbohydrate & Energy Metabolism During Follicle Growth & Oocyte Maturation

What Can Be Learned From IVM & Follicle Culture?

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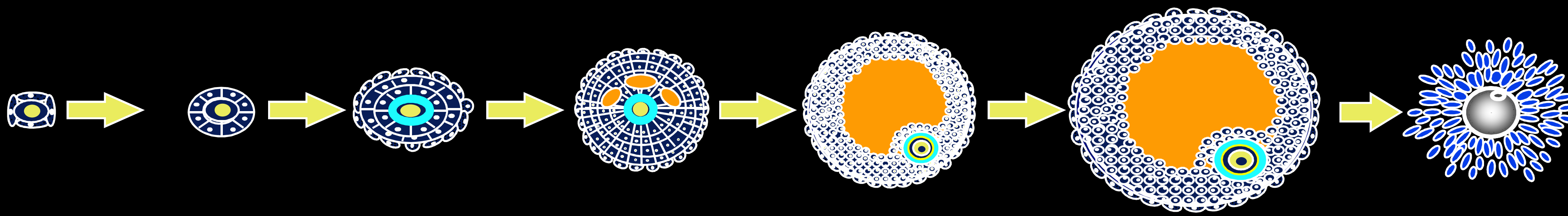
The Biology Of Follicle & Oocyte Development

Primordial follicle (30-40 µm) | Primary follicle (50 µm) | Secondary follicle (50-200 µm) | Pre antral-antral follicle (>220 µm) | Early antral-antral follicle (0.5-2.0 mm) | Graafian follicle (2.0- ≥17 mm) | Ovulation



A-cyclic recruitment | Cyclic recruitment | Selection

Gonadotrophin responsive

Gonadotrophin dependent

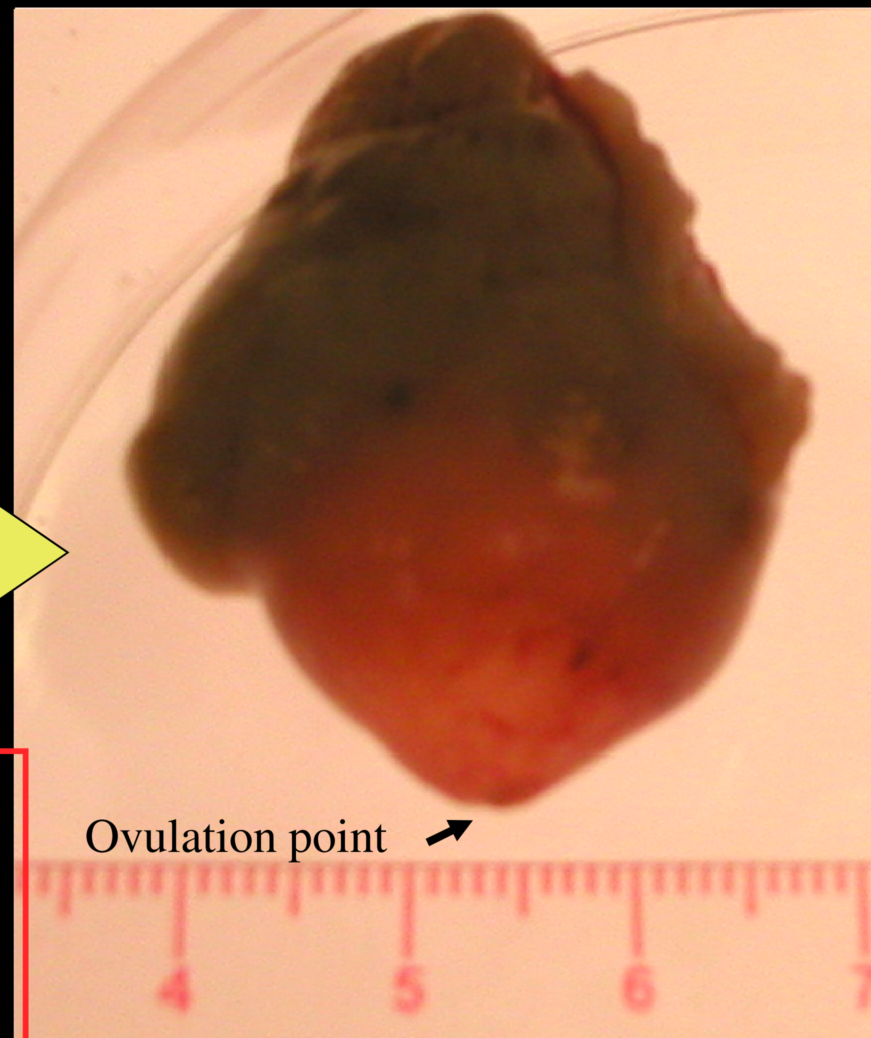
Molecular regulation: transcription factors, growth factors, peptides, steroids

Cell-cell interactions & signalling for follicle & oocyte growth & development

Follicular Fluid And Granulosa Cell Markers Of Follicle And Oocyte Development

Nutrients
Growth factors
Hormones
Oxygen tension
Granulosa cells

1.5 mm Antral Follicle



18 mm Ovulatory Follicle

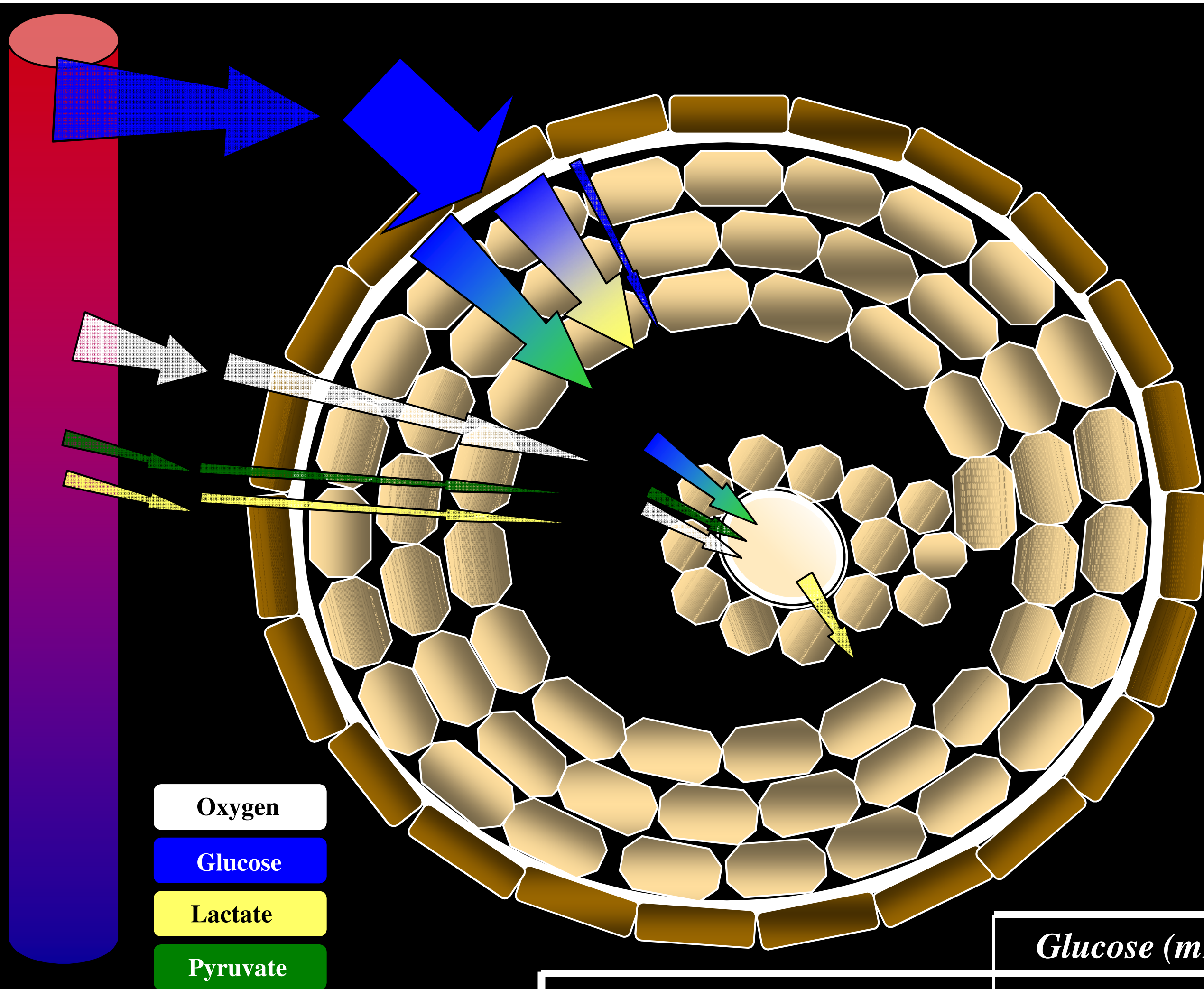
Metabolic requirements change as:

i) Follicle size and vascularity ⇧

ii) Follicle differentiation ⇧

iii) Follicle exposure to LH/ hCG

Nutrient Origin & Consumption During Follicle & Oocyte Growth *In Vivo*



	Glucose (mM)	*Pyruvate (mM)*	*L-Lactate (mM)*
Follicular fluid (mouse)	0.46	0.38	17.3
Oviduct fluid (mouse)	1.09 (+ *CCs*)	0.37 (+ *CCs*)	10.9 (+ *CCs*)
Plasma (mouse)	11.7	0.16	4.8
Follicular fluid (human)	3	0.26	6.06
Oviduct fluid (human)	0.5-3.11	0.24-0.32	4.87-10.55
Plasma (human)	5	0.1	0.6

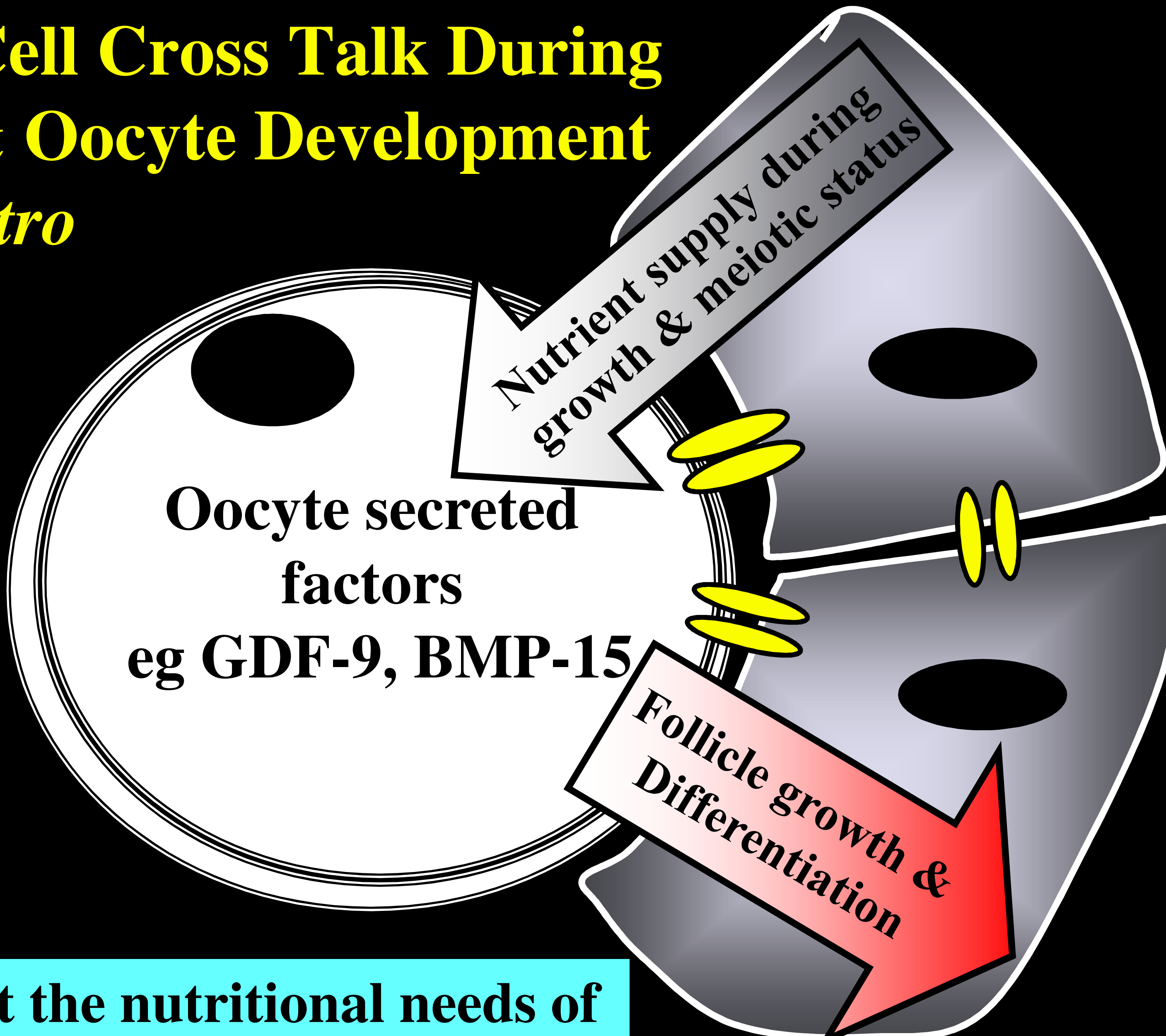
Adapted from: Harris & Picton, 2007

Nutrient Composition Of Body Fluids

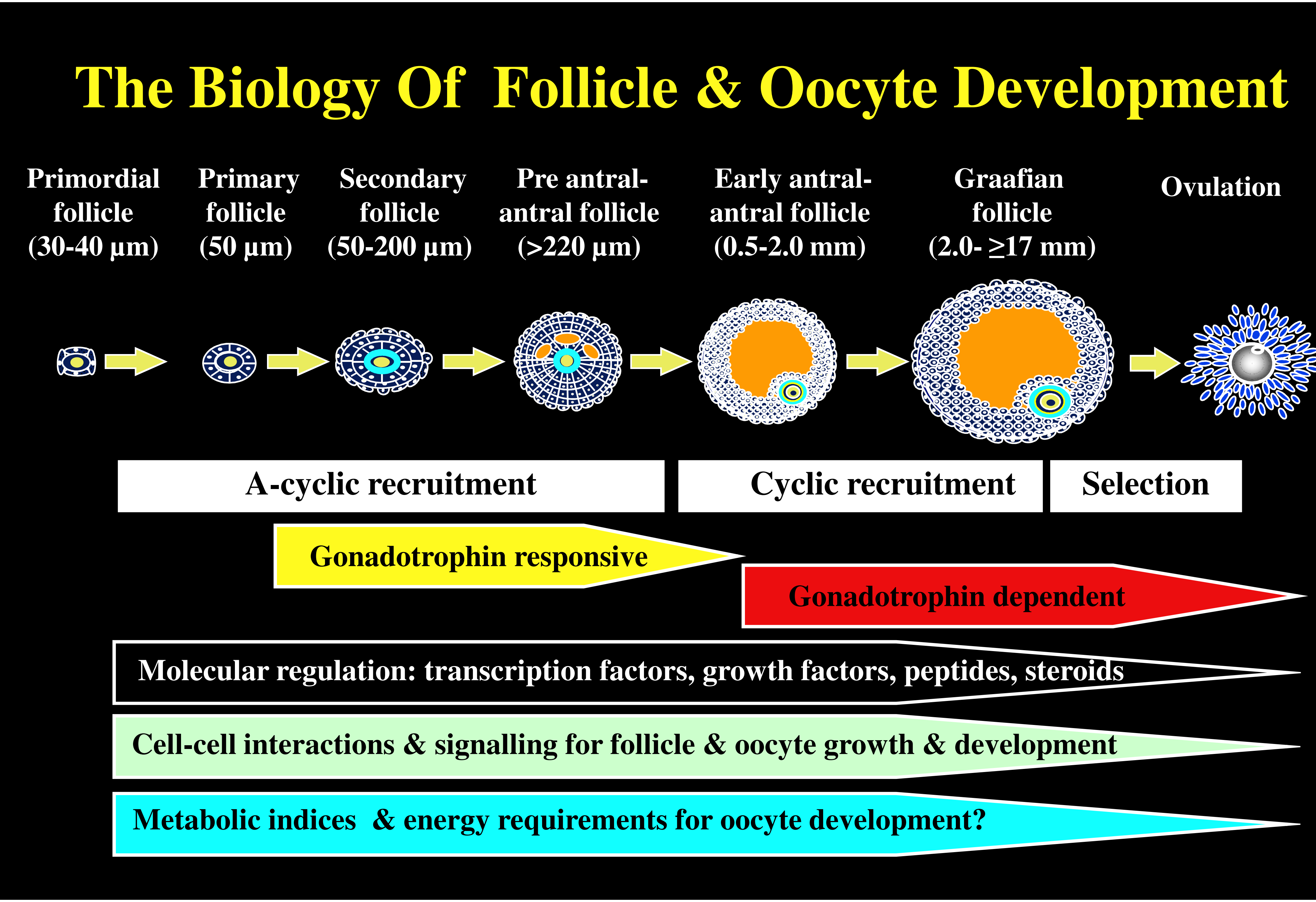
Fluid Source	Glucose (mmol l^{-1})	Lactate (mmol l^{-1})	Pyruvate (mmol l^{-1})	Species
	3 - 3.39	3.17- 6.06	0.26	Human (Leese & Lenton, 1990; Gull et al. 1999)
	0.46	17.3	0.38	Mouse (Harris et al. 2005)
	4.8	5.	0.03	Cow (Orsi et al. 2005)
Oviduct	0.5 - 3.11	4.87 - 10.55	0.24	Human (Gardner et al. 1996)
	0.53 - 1.1	5.4 - 8.58	0.14 - 0.17	Human (Tay et al. 1997, Dickens et al 1995)
	0.59	5.71	-	Pig (Nichol et al. 1992)
	0.07	-	-	Cow (Carlson et al. 1970)
	1.65	11.7	0.17	Mouse (Harris et al. 2005)
Plasma	5	0.6	0.1	Human (Borland et al. 1980)
Blood	3.5 – 11.7	0.56 - 5.64	0.16-0.18	Mouse (Wang et al. 2003, Sanni et al. 2001, Harris et al. 2005)

Oocyte-Somatic Cell Cross Talk During Normal Follicle & Oocyte Development *In Vivo* And *In Vitro*

Bidirectional communication between the oocyte & GCs via gap junctions



Gap junctions support the nutritional needs of follicle & oocyte growth *in vivo* & *in vitro?*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Metabolic indices & energy requirements for oocyte development?

Metabolism Measurement During Follicle And Oocyte Development *In Vivo And In Vitro*

Uptake From media

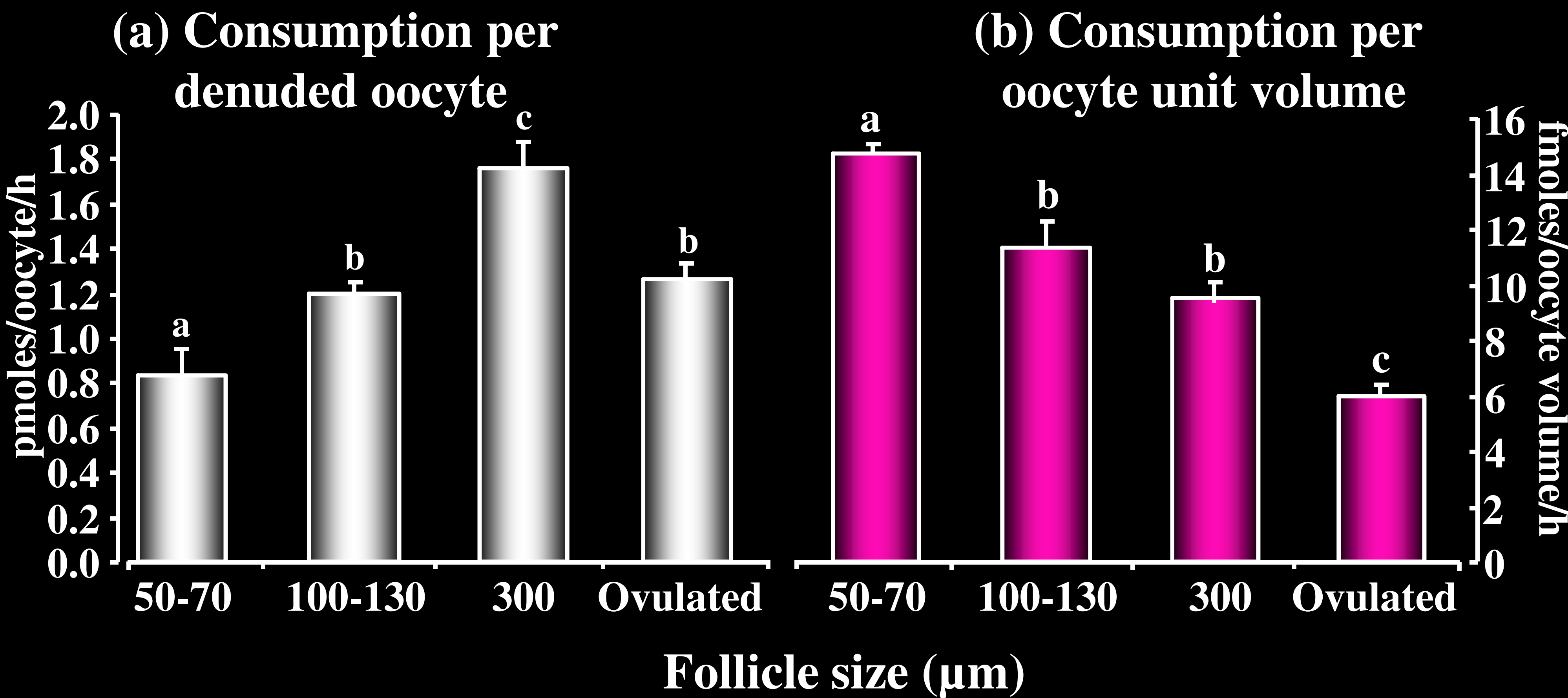
- Pyruvate
- Glucose
- Lactate
- Lipid
- Amino acids
- Oxygen

Production Into media

- $H_2 0$
- CO_2
- Lactate
- NH_4^+
- Amino acids
- Enzymes
- Growth factors /peptides

Influenced by stage of follicle/oocyte development and differentiation

Pyruvate Consumption By Individual Oocytes Throughout Mouse Oocyte Development



Different letters are significantly different at $p<0.05$

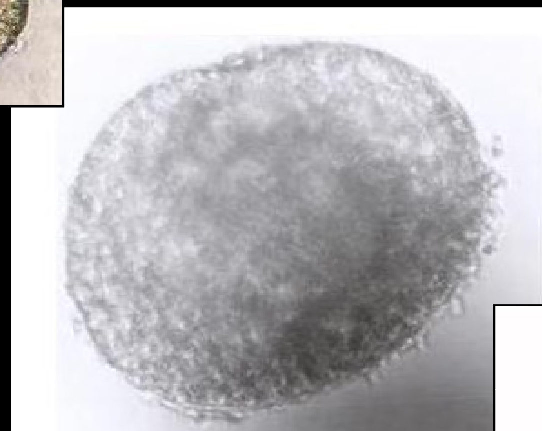
Harris et al (2009) Mol Reprod Dev. 76:231

Mouse Follicle Metabolism *In Vivo*

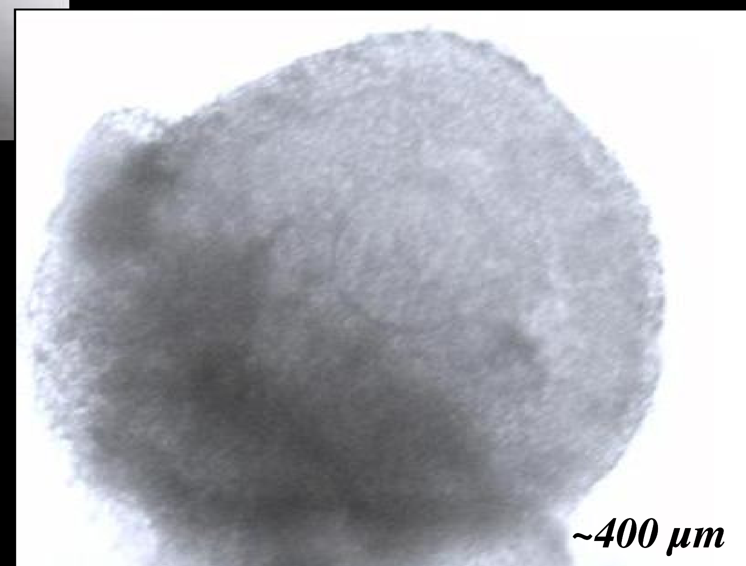
Small follicles use a combination of glycolytic & aerobic metabolism of glucose

<18 µm

Diffusion of nutrients across small distances: primordial follicles utilise a variety of carbohydrate energy substrates



Large follicles become almost totally reliant on glycolytic glucose consumption

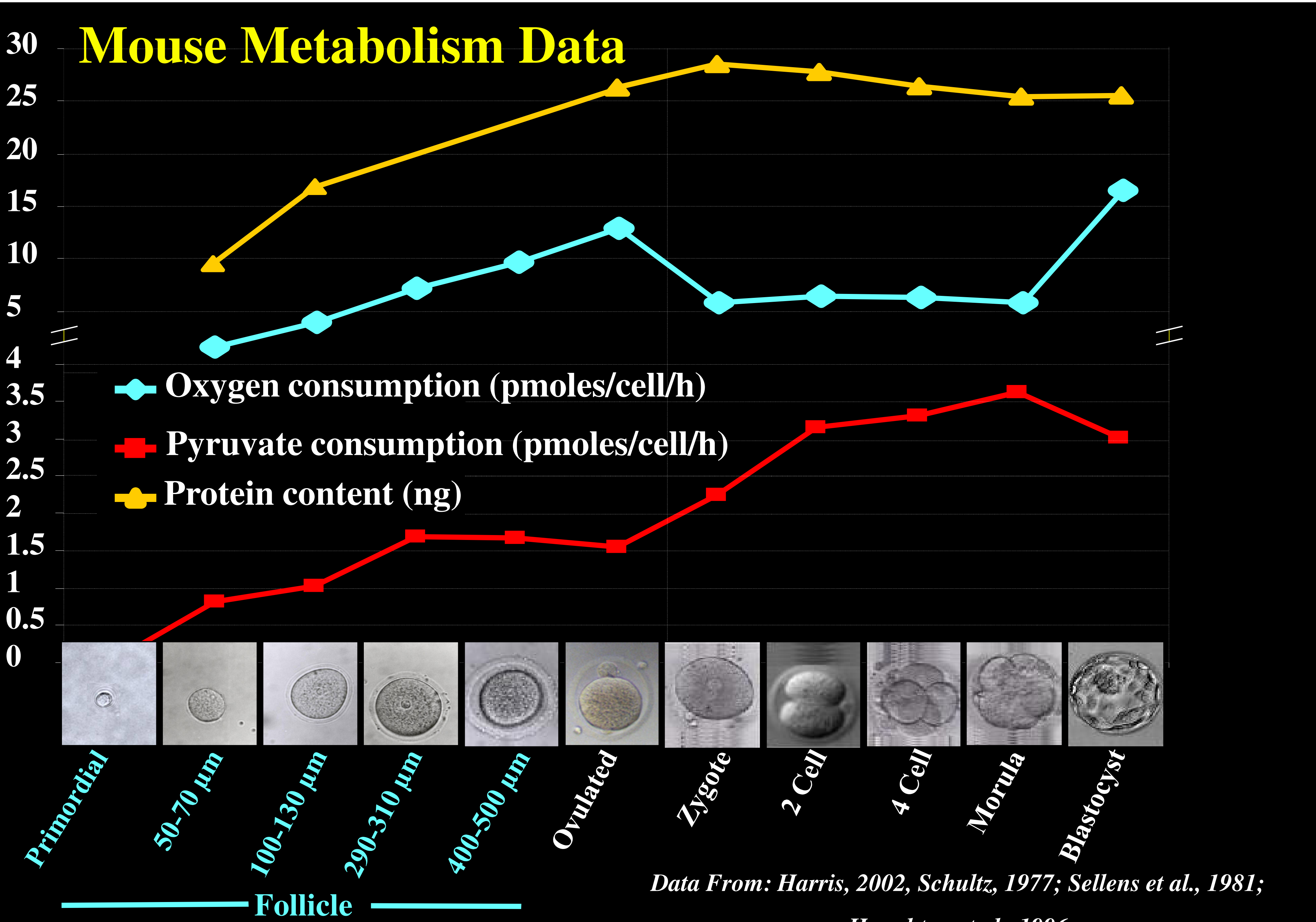


Primordial – Graafian follicle

Mouse: >30,000 –fold increase in volume

Human: >91,000,000 –fold increase in volume

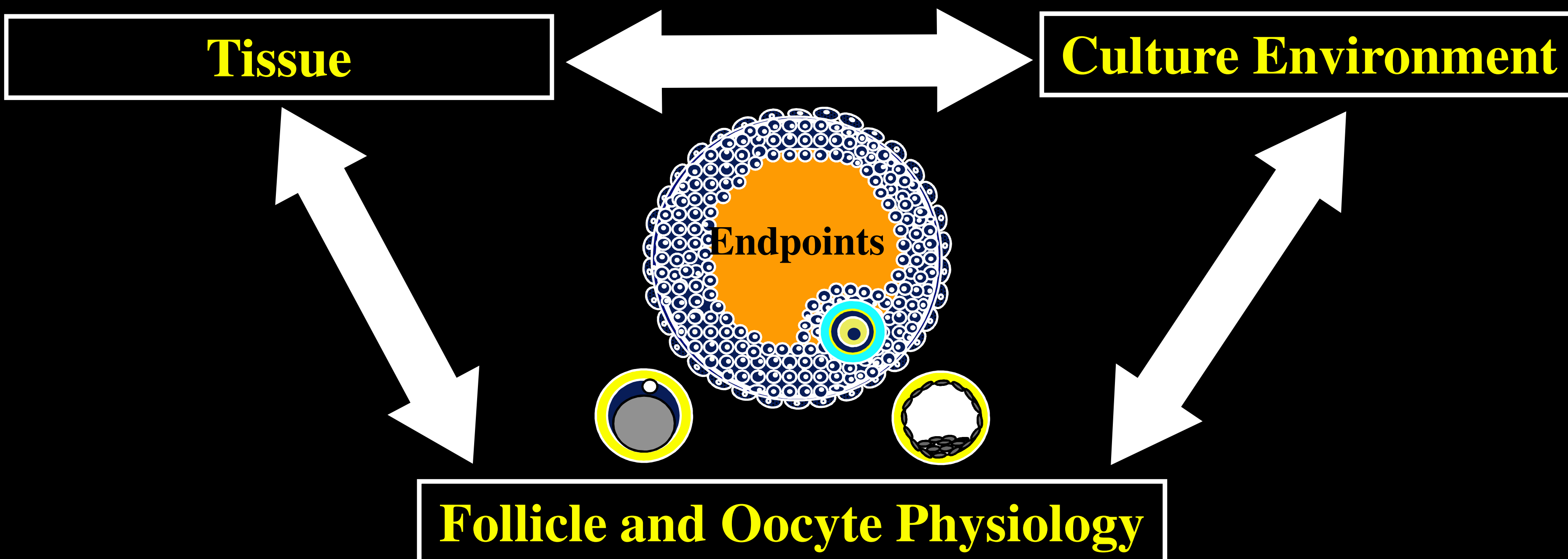
(Harris 2002, Harris et al., 2007)

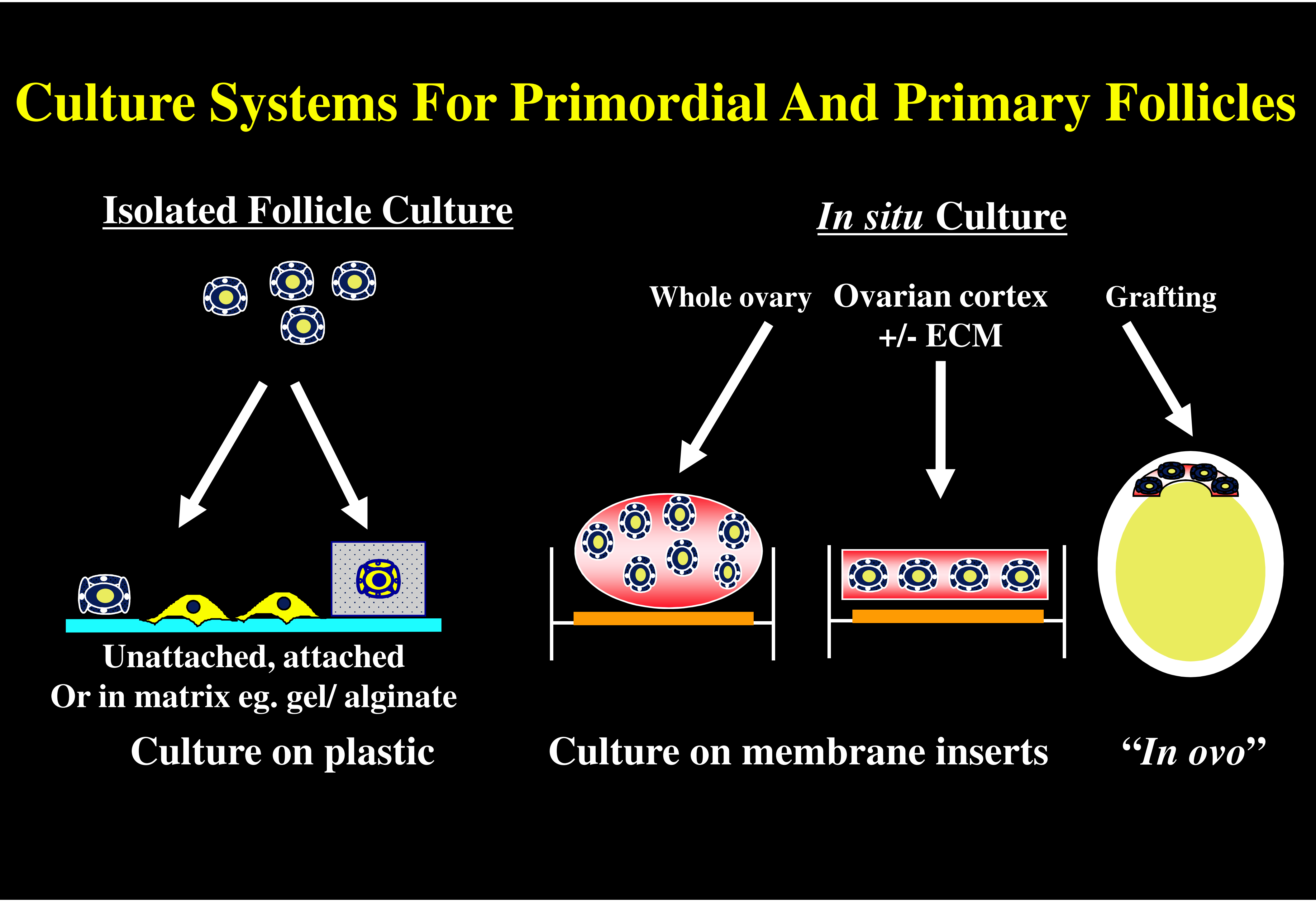


Data From: Harris, 2002, Schultz, 1977; Sellens et al., 1981; Houghton et al., 1996

What Can Be Learned From IVM & Follicle Culture?

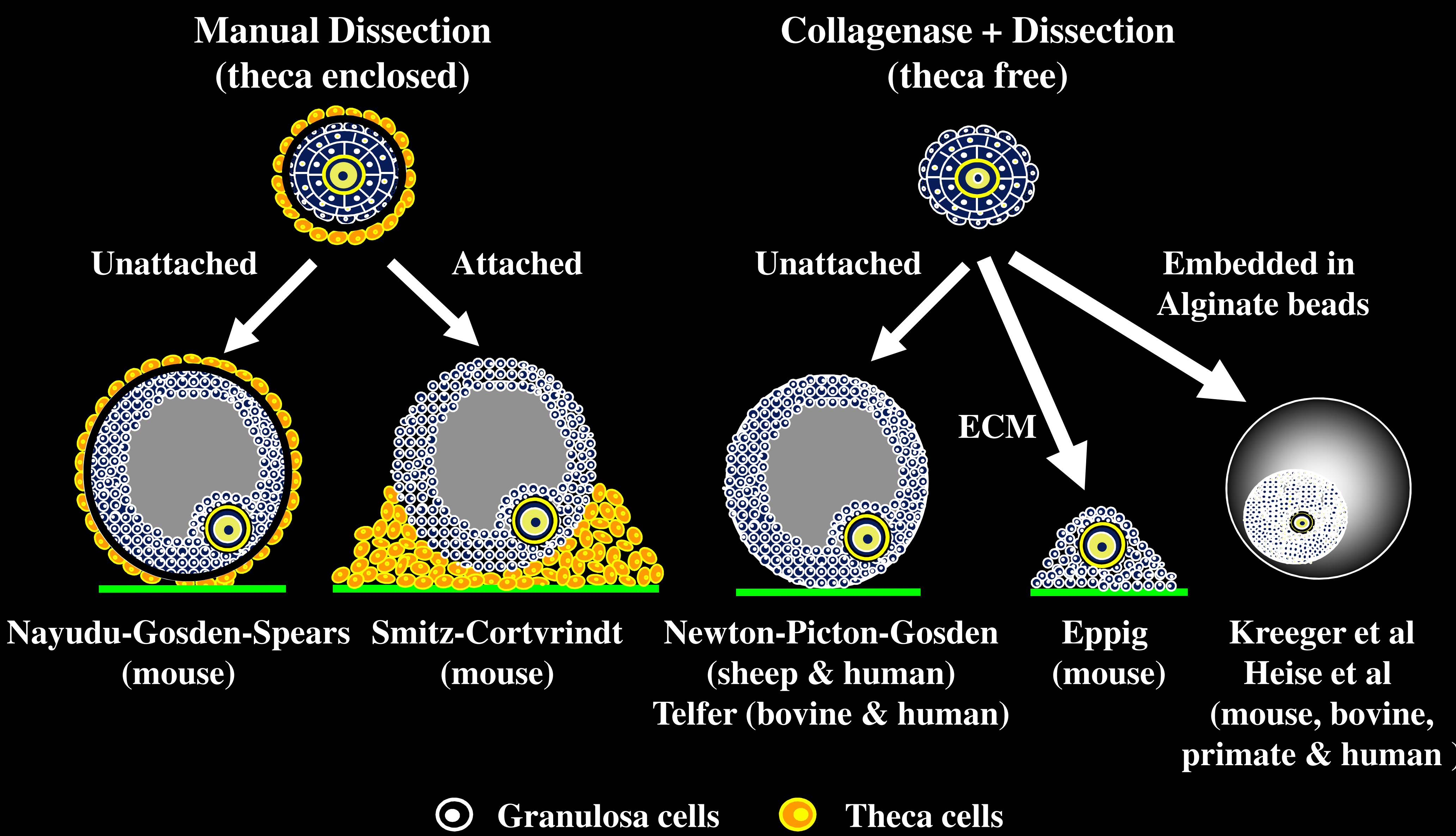
Rodent, Ruminant And Human





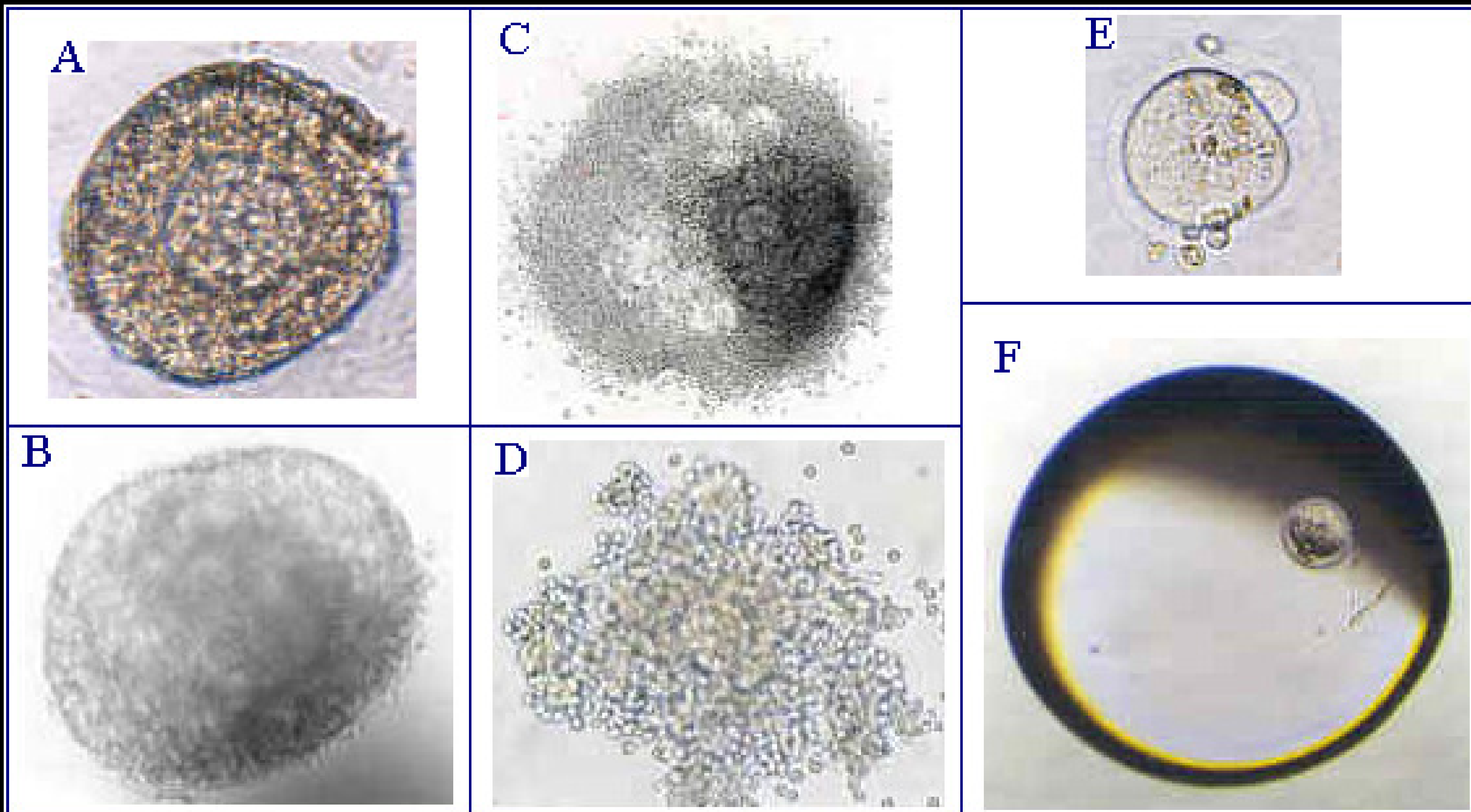
Culture Systems For Primordial And Primary Follicles
Isolated Follicle Culture
In situ Culture
Whole ovary
Ovarian cortex
+/- ECM
Grafting
Unattached, attached
Or in matrix eg. gel/ alginate
Culture on plastic
Culture on membrane inserts
"In ovo"

Culture Systems For Preantral Follicles



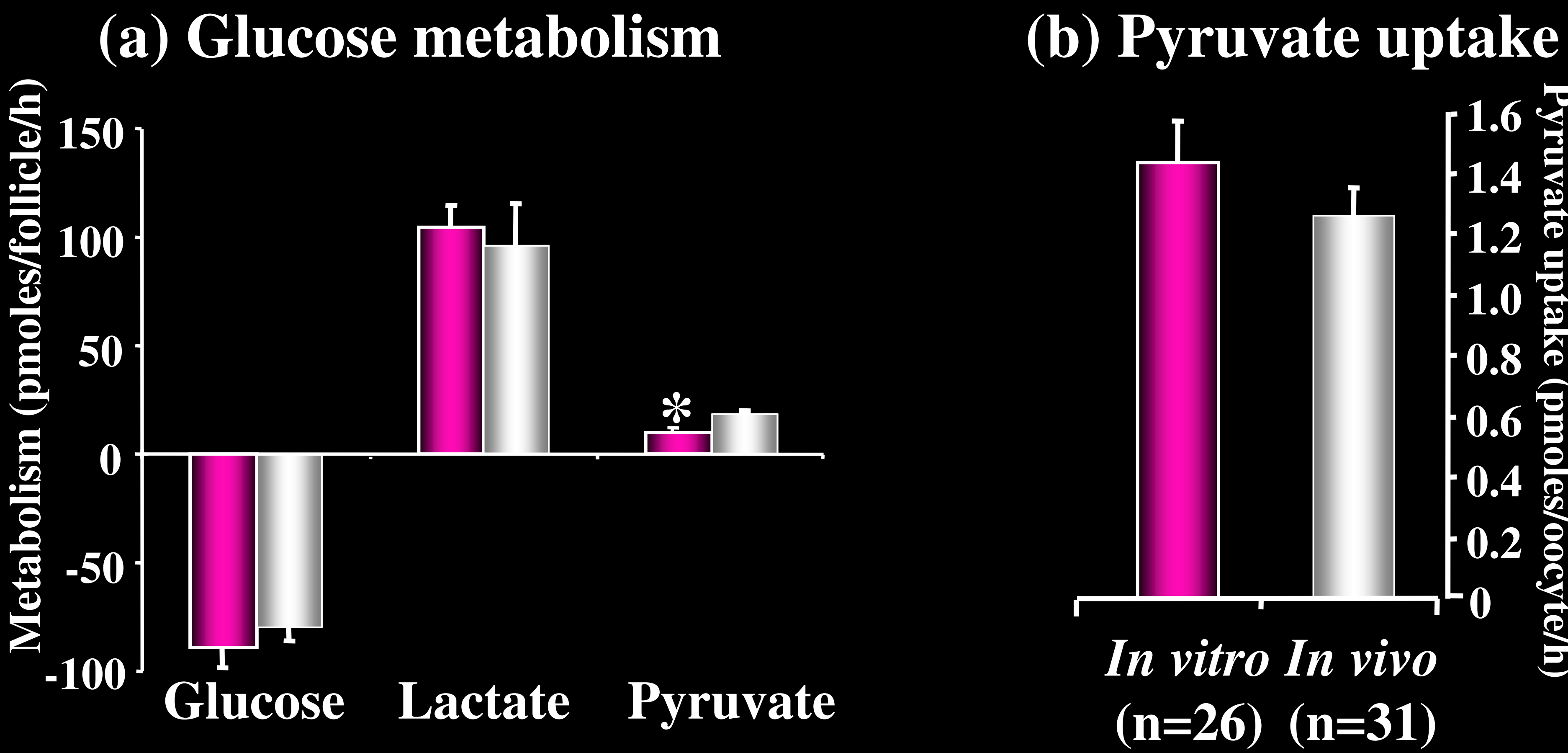
In Vitro Grown Mouse Oocytes

(Follicles cultured according to the method of Cortvrindt & Smitz, 1996)



Harris et al 2007 Reproduction. 134:415

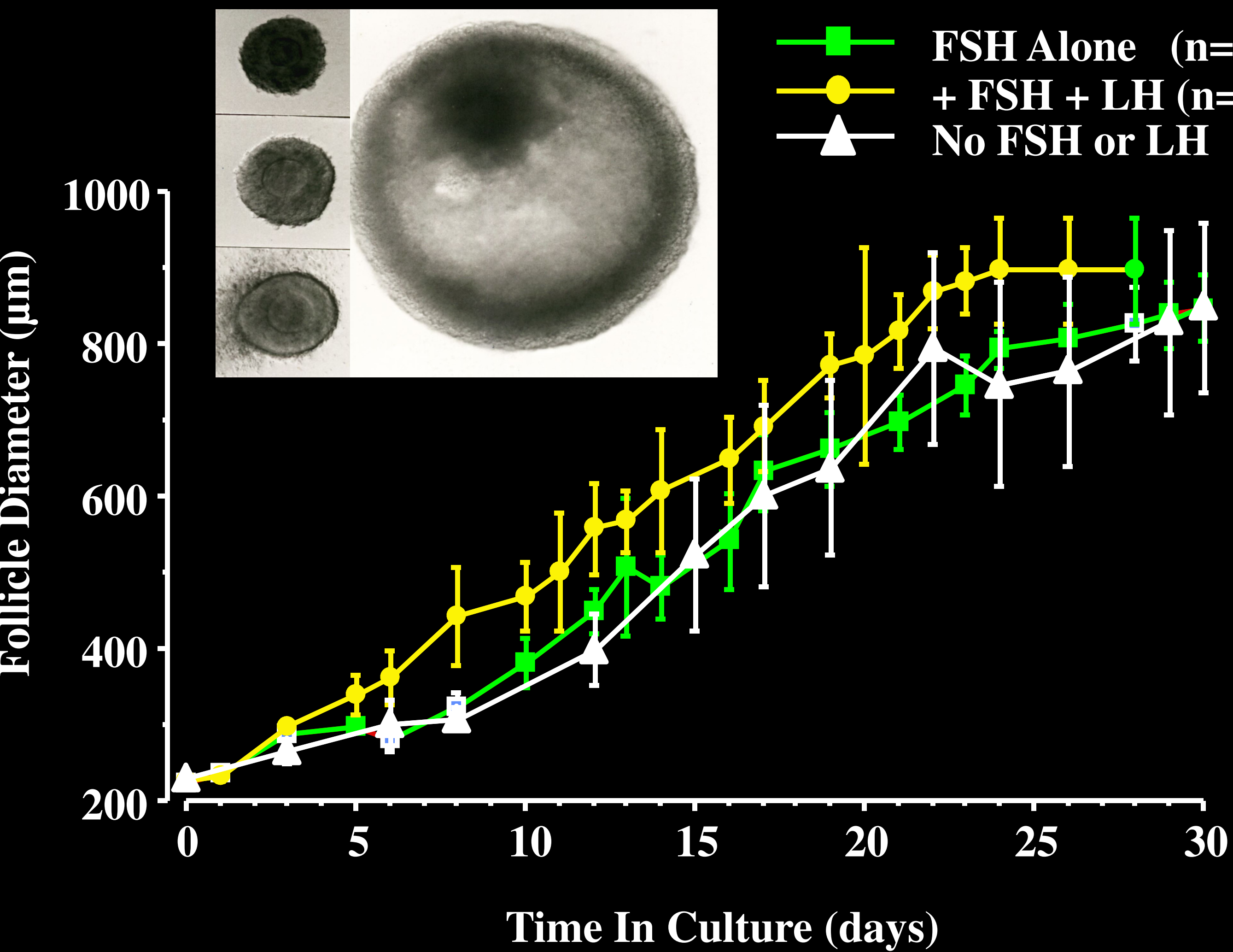
Energy Metabolism By Individual Metaphase II Mouse Oocytes Grown *In Vivo* & *In Vitro*

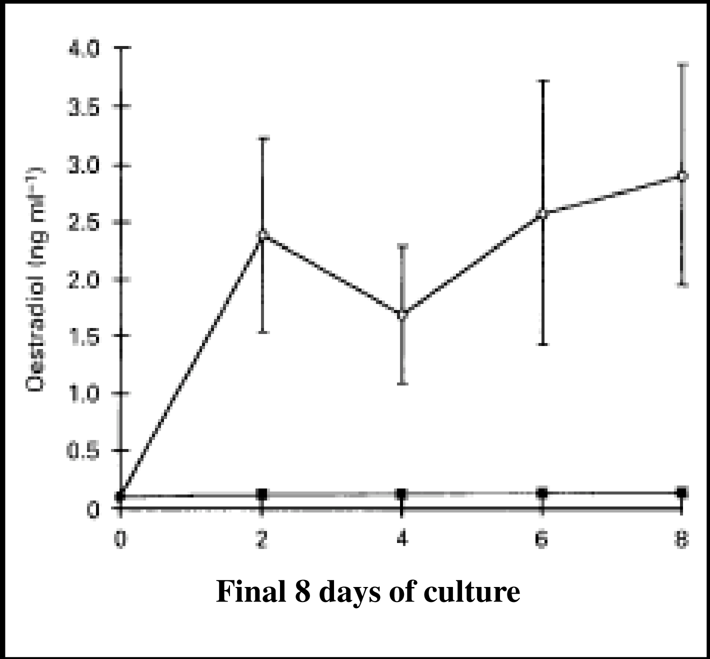


Harris et al 2007 Reproduction. 134:415

Gonadotrophic Regulation Of Preantral Ovine Follicle Growth *In Vitro*

(Preantral follicles: 180-200µm diameter)



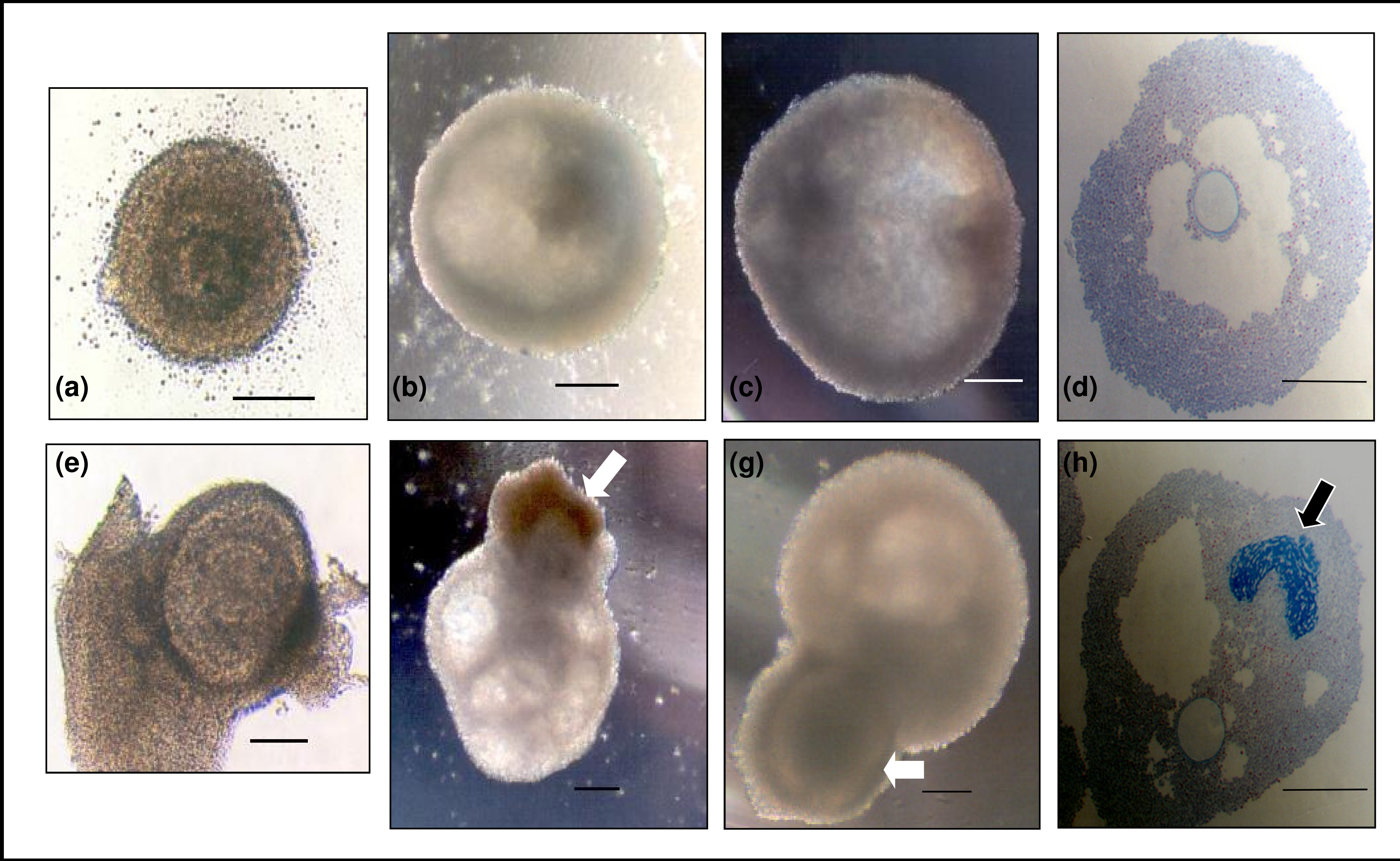


(Newton et al., 1999)

Manipulation Of *IVG* of Sheep Preantral Follicles

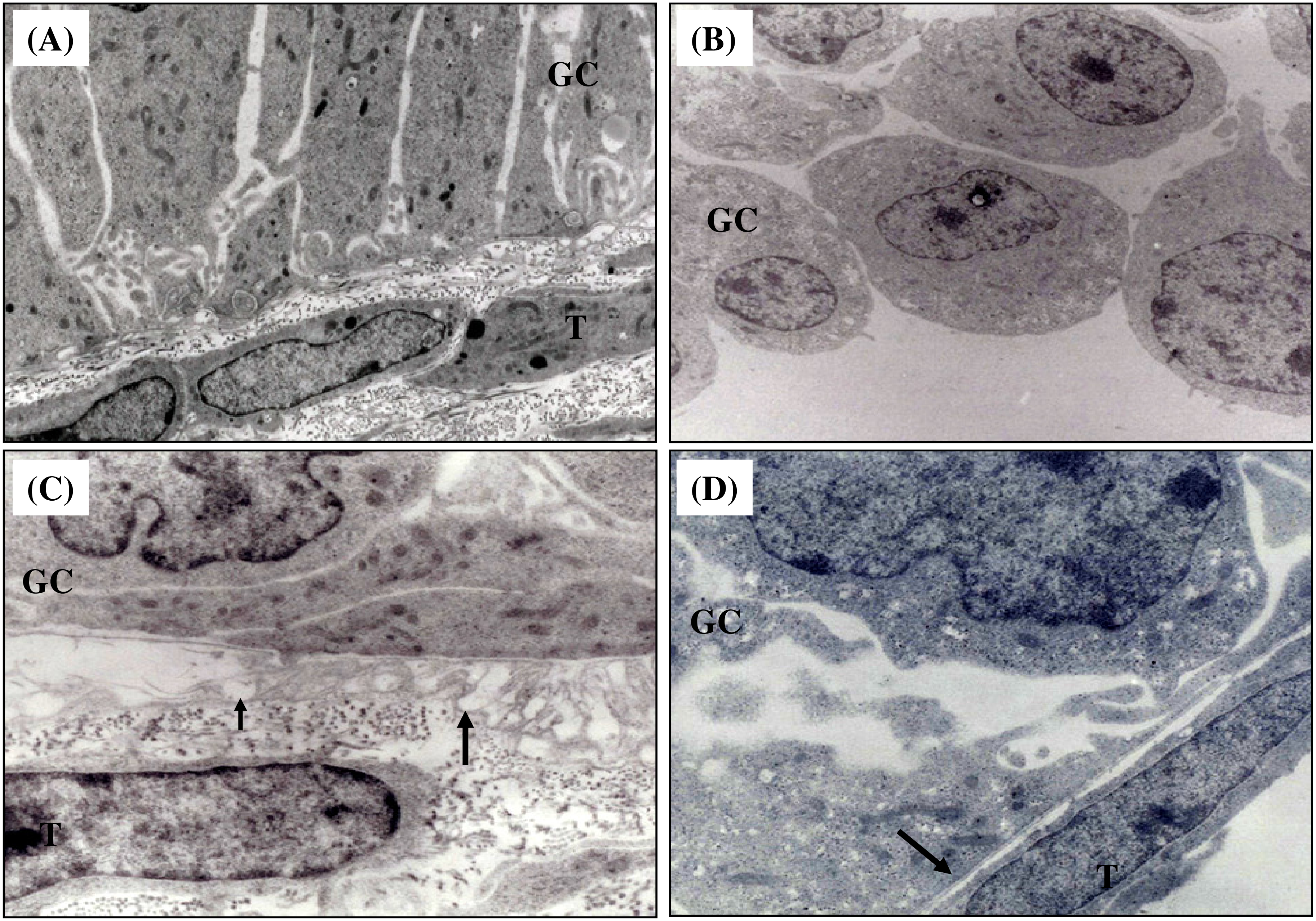
(a) - (d) theca-free follicles harvested by enzyme digestion

(e) - (h) theca-enclosed follicles harvested by mechanical isolation



Picton et al (2003) Reprod Suppl. 61.

TEMs Of Sheep Follicles Cultured For 30 Days



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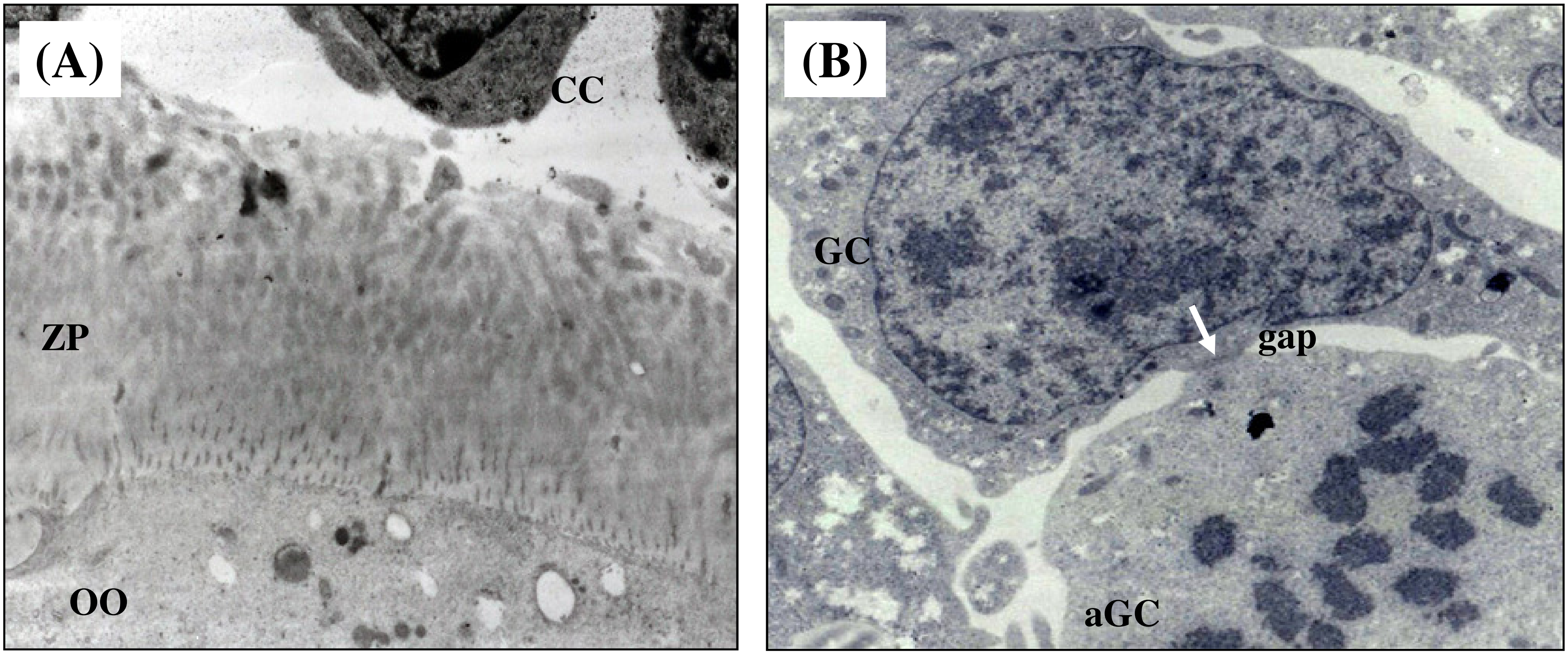


Follicles cultured for 30 days in serum-free medium containing ascorbate

Picton et al 2006

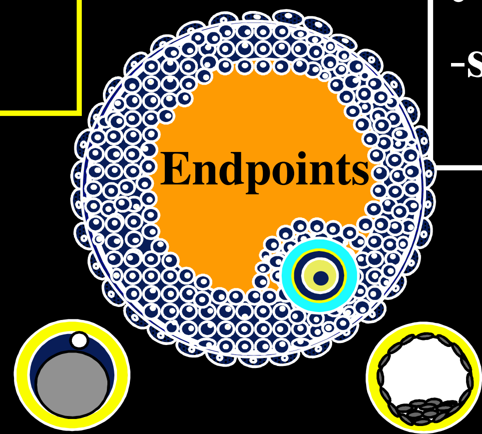
Summary Of Metabolism Measurements During *In Vitro* Growth Of Follicles and Oocytes

Oocyte Physiology

Growth rates
Growth initiators & regulators
Gene transcription & translation
Epigenetic regulation
Metabolic requirements

Culture Environment

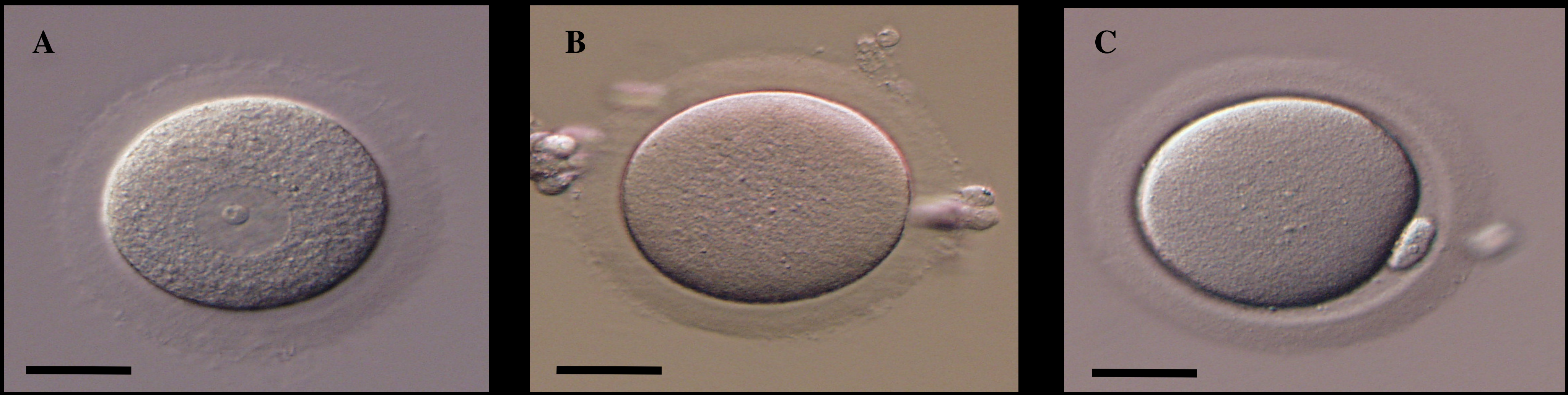
- Media composition
  - Growth factors?
  - Substrates & energy?
  - Paracrine production?
- Culture system & duration
  -sequential, perfusion, 0_2



Metabolic indices & energy requirements for oocyte development *in vivo*

In Vitro Maturation Of Oocytes

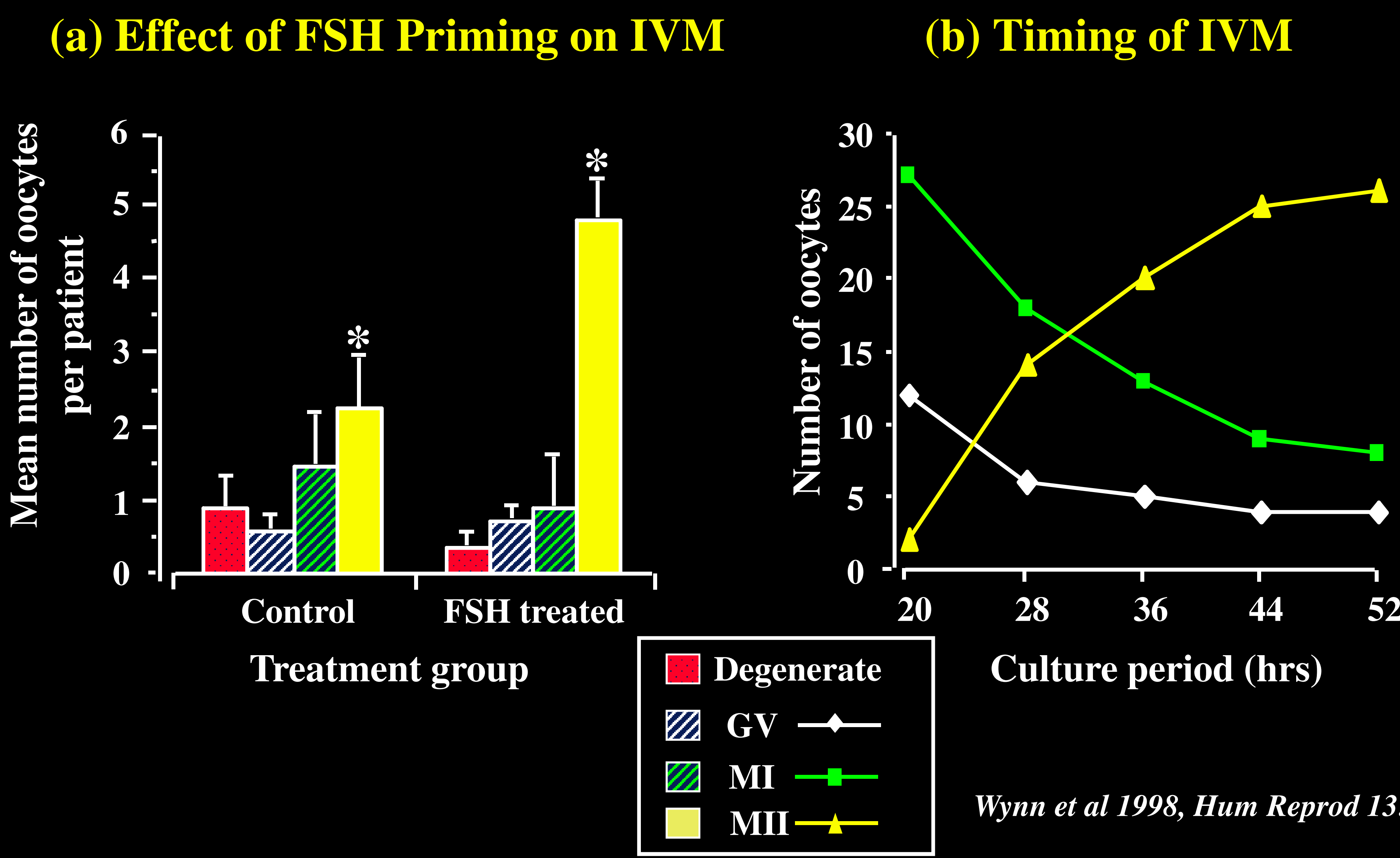
The *In Vitro* Maturation Of Oocytes



Results in all species indicate that we can:

- Induce nuclear and cytoplasmic maturation in cumulus enclosed secondary oocytes to produce MII gametes with variable developmental potential
- More limited understanding of the developmental potential and epigenetic normality of oocytes derived from early stages *in vitro*.
- Clear need to improve efficiency of IVM by quantification of the biological risks and limitations of the technology

Analysis of Oocyte Maturation *In Vitro* In Humans



Wynn et al 1998, Hum Reprod 13: 3132

Effect Of Gonadotrophins During IVM On Ovine Embryo Development *In Vitro*

IVM Media	No oocytes inseminated	Embryo cleavage no.	Blastocyst no.
Serum-free + Gn	146	103 (70.5%)	29 (28.1%)
Serum-free - Gn	112	59 (52.6%)*	4 (6.7%)*
10% FCS + Gn	139	105 (75.7%)	24 (22.8%)

* = P< 0.05

Cotterill, Catt & Picton Unpublished

Can Amino Acid Turnover And Energy Metabolism Be Measured In Individual Oocytes ?

?

1. **Do developmentally competent oocytes have a distinct metabolic finger print ?**
2. **Can the metabolic signature of an oocyte be linked to molecular &/or cytogenetic correlates of developmental competence ?**

Physiological Functions Of Amino Acids

- Building blocks for protein synthesis
- Energy source
- Involved in nucleotide synthesis
- Osmolyte functions
- Antioxidant functions
- Involved in pH regulation (micro buffer function)
- Chelators- working as protection against oxidation
- Signalling molecule precursors

Amino Acid Profiling
As The Means To Select The Best Embryo

Philosophy of Approach

- **The most viable preimplantation embryos are those with the lowest level of metabolism i.e. the *"quiet embryos"***

  overall metabolism, aa turnover and glycolysis

- **Low metabolism is achieved by reducing the concentration of nutrients in culture media to the levels measured in the female reproductive tract, this encourages the embryo to use endogenous resources.**

Can this approach be applied to individual oocytes?

Evidence of links between oocyte metabolism & quality during IVM in cows

What about humans?

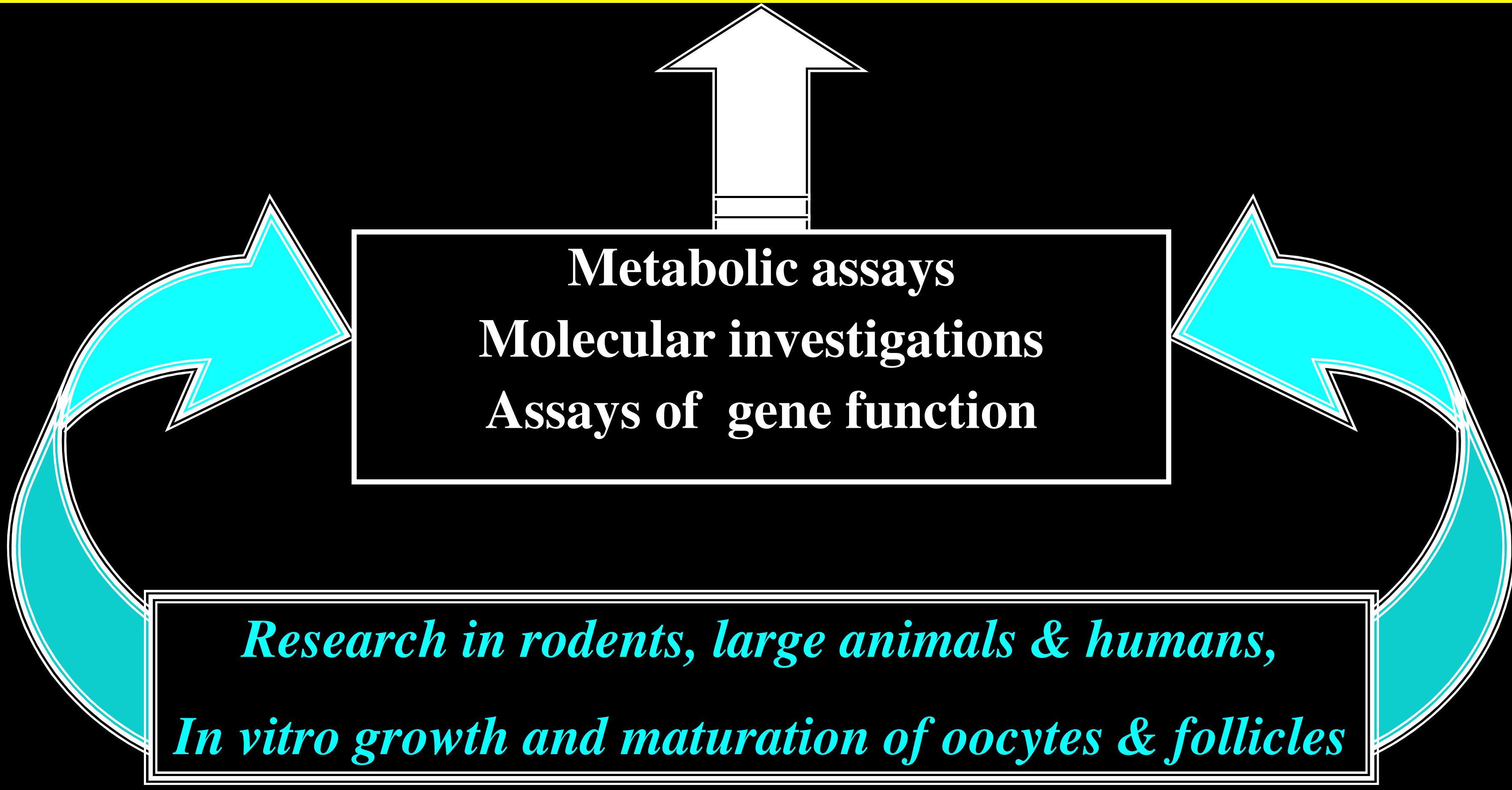
Summary Of Metabolic Studies Of The Later Stages Of Oocyte Development During IVM

- Amino acid consumption/production is significantly different between individual, developmentally competent bovine MII oocytes and those which fail to fertilise and/or arrest during embryo cleavage.

- Asparagine, glutamine, serine and phenylalanine turnover are potential markers of bovine oocyte developmental competence.

- Carbohydrate and amino acid metabolism by human oocytes are significantly linked to oocyte developmental competence, patient age, aetiology, gonadotrophin dose/treatment and insulin sensitising drug treatment *in vivo*.

Follicle and Oocyte Development In Health & Disease



Acknowledgements

ReD Labs

John Huntriss
Jan Hogg
Emma Chambers
Sally Catt
Sarah Harris
Liming Gui
Wanziri Muruvi
Jianping Lu
Ping Jin
Matthew Hinkins
Karen Hemmings
Matthew Cotterill
Morag Gunsen
Esther Collado

Leeds NHS Trust

Adam Balen
Vinay Sharma
Anthony Rutherford
Adam Glaser
Tommy Tang
Ephia Yasmin
Manisha Palep Sing
Vickie Rawnsley
Deivanayagam Maruthini

Collaborators

Bruce Campbell
Martin Luck
Morag Hunter
Bob Webb
Henry Leese

Sponsors

Serono, Novo Nordisk, LGI Special Trustees, BDF, Candlelighters, BBSRC, MRC, BFS