Glycolytic activity as a tool for embryo selection

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„Fight of the Queens“

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INTRODUCTION

- **Definition**:

  The totality of the chemical processes that an organism or cell is capable to perform.

Mahler & Cordes, Biological Chemistry 1971, Harper International Edition, N.Y.

INTRODUCTION

- Catabolic reactions & sequences
- Anabolic reactions & sequences
- Anaplerotic reactions & sequences

INTRODUCTION

Catabolic routes:

Degradative processes in which large organic molecules are broken down to simply cellular constituents, with attendant release of chemical free energy.

INTRODUCTION

Anabolic routes:

Synthetic processes that produce complex organic cellular constituents from simpler precursors, frequently involving reductive steps and require the expenditure of chemical free energy.

INTRODUCTION

Anaplerotic routes:

Ancillary sequences that involve the insertion of either a 1 Carbon (CO_2) or a 2 Carbon fragment acetyl CoA) into the common pool from which anabolism drains constantly.

INTRODUCTION

CENTRAL PATHWAYS

Carbohydrates

Triose phosphates and/or pyruvate

Fats

Acetyl CoA, propionyl CoA and glycerol

Proteins

Acetyl CoA, oxalacetate

α-oxoglutarate,fumarate and succinate

INTRODUCTION

CRUCIAL INTERMEDIATES

– Triose phosphate – pyruvate - acetyl CoA

– Oxalacetate – aspartate, α-oxoglutarate – glutamate

– Complete cyclic combustion of actetyl CoA to CO_2 and H_2O (Citric Acid Cycle)

INTRODUCTION

GOAL

– To develop an artificial mixture of known chemical components that can substitute for the natural microenvironments encountered by an embryo as it develops from the one cell zygote to the blastocyst.

J.D. Biggers (1998) Int. J. Dev. Biol.42, 879-884

Glycolytic Activity

INTRODUCTION

GOAL

- Specific Parameter(s)
  - Reflecting Health Competent of the Embryo
- Non-Invasive Method
  - User Friendly
  - Accurate & Precise

INTRODUCTION

LIMITATIONS

- There are no preparations for the study of embryo metabolism in situ or in vivo, such as exist for large vascularized tissues.
- For this reason it remains an act of faith that metabolism of the embryo in vitro reflects that in the female tract.

H.J. Leese Oxford Reviews of Reproductive Biology (1991) 13, 35-72

MORE THAN HALF A CENTURY OF METABOLIC STUDIES

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Early Pioneers

1949 Hammond J.

- Physiological Saline Hen egg white and yolk: mouse 8-cell stage to blastocyst.

1956-57 Whitten

- Krebs Ringer bicarbonate physiological saline, with crystalline BSA, Ca-lactate.

1958 Mc Laren & Biggers

- Normal offspring in mouse with Whittens medium.

Early Pioneers

1965 Brinster

- Glucose is not possible as sole energy substrate for the mouse
  - (pyruvate, phosphoenolpyruvate, oxalacetate as substitute for lactate)

1976 Wordinger & Brinster

- Glucose is necessary for blastocyst formation

Proposed –Studied Parameters

- Glucose
- Pyruvate
- Lactate
- Glutamate
- Amino Acids
- Fatt.

GLUCOSE

- **Energy Source**

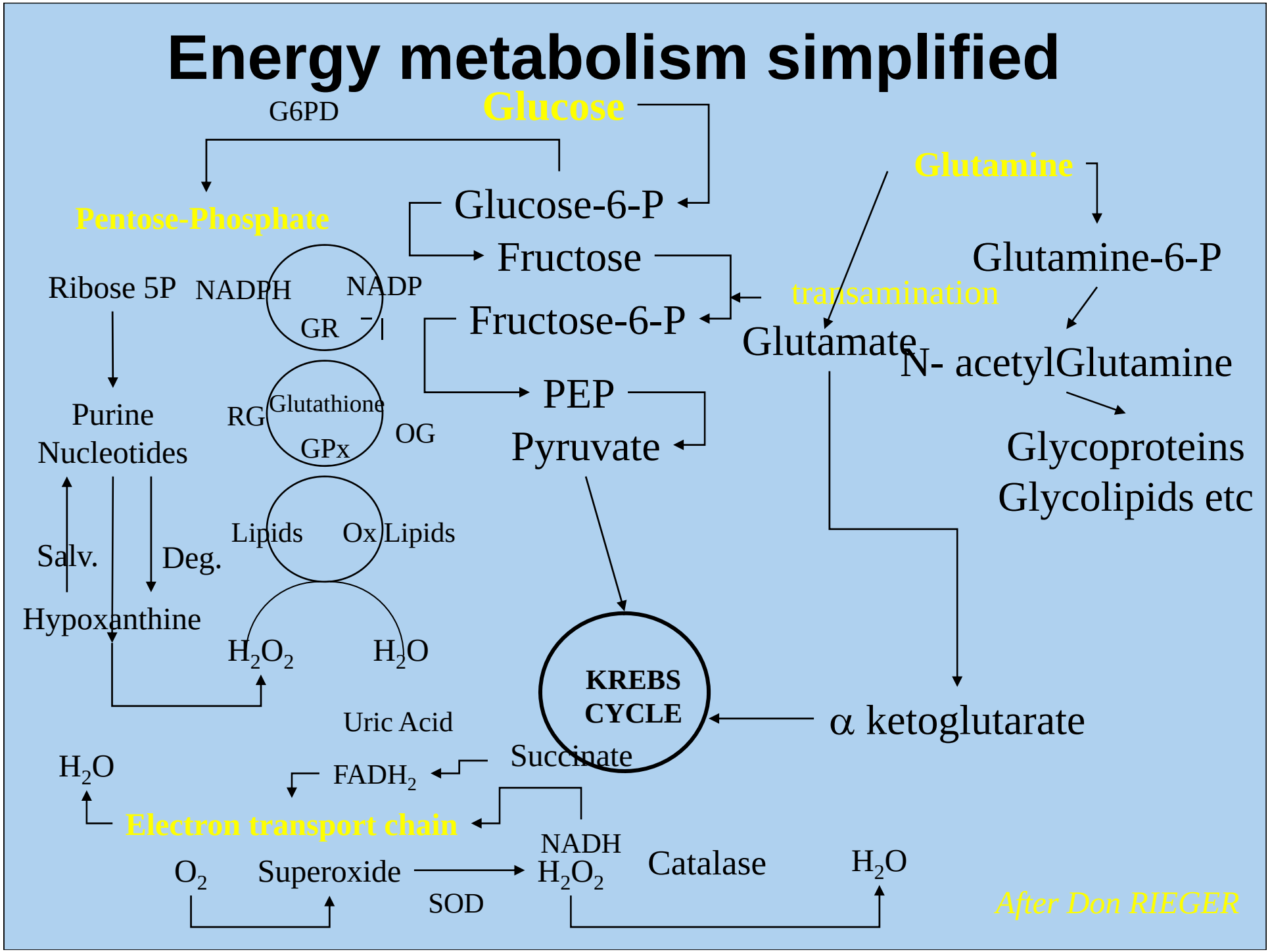
- **Key Anabolic Precursor**

  - Synthesis
    - Triacylglycerols
    - Phospholipids
    - Mucopolysaccharides & glycoproteins
    - Ribose moieties, NADPH (lipids, glutathione)

Respiration - Glycolysis

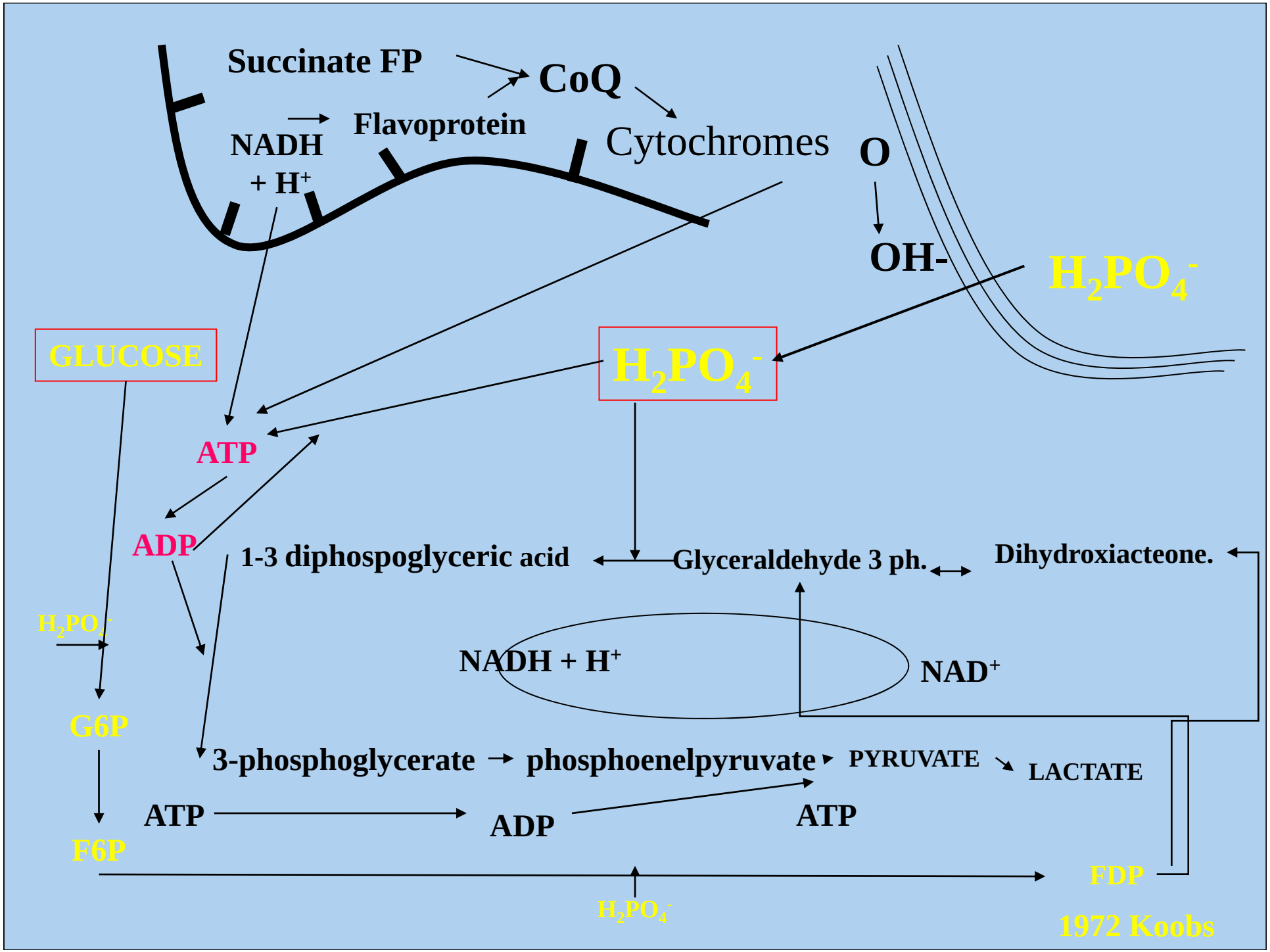
- **Krebs –TCA-cycle**
  - Glucose
  - Pyruvate de-carboxilation
  - Acetyl CoA
  - 38 ATP
  - Electron transport
    - Superoxide anion
    - H_2O_2
- **Embden-Meyerhof**
  - G-6-P
  - 2 Pyruvates
  - 2 ATP

Energy metabolism simplified



Crabtree Effect

- Manifistation of Respiratory Inhibition after addition of glucose or a hexose capable of being phosphorylated by hexosekinase.
- Competition between glycolysis and oxidative phosphorylation for ADP and Phosphate.
- Glycolysis does not remain inhibited but increases to a steady state until all the glucose is phosphorylated


Succinate FP
CoQ
Flavoprotein
NADH
+ H+
Cytochromes
O
OH-
H2PO4-
GLUCOSE
H2PO4-
ATP
ADP
1-3 diphospoglyceric acid
Glyceraldehyde 3 ph.
Dihydroxiacteone.
H2PO4-
NADH + H+
NAD+
G6P
3-phosphoglycerate
phosphoenelpyruvate
PYRUVATE
LACTATE
ATP
ADP
ATP
F6P
FDP
H2PO4-
1972 Koobs

Glycolytic activity as a selection tool ?

- Emden-Mayerhof-pathway
  - 1 Glucose converted to 2 lactate

- Used in Cattle and mouse to select embryos embryos with high implantation.
  - Renard JP. et al 1980.
  - Gardner D. et al. 1996.

Microfluorometric Assays

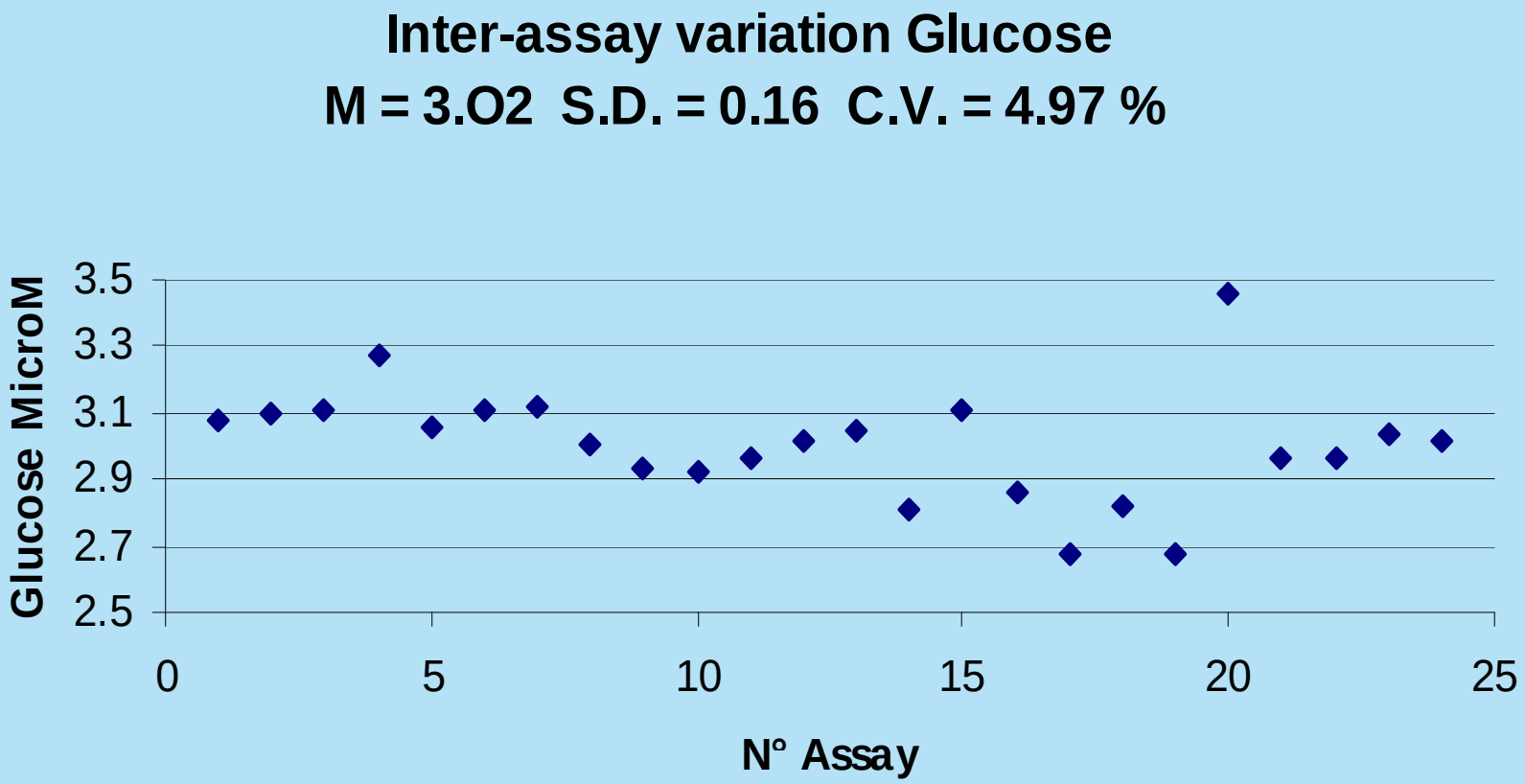
$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{glucose-6phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + \text{NADP}^+/\text{NADPH} + \text{H}^+ \xrightarrow{\text{G6PDH}} \text{6-phosphogluconate}$$

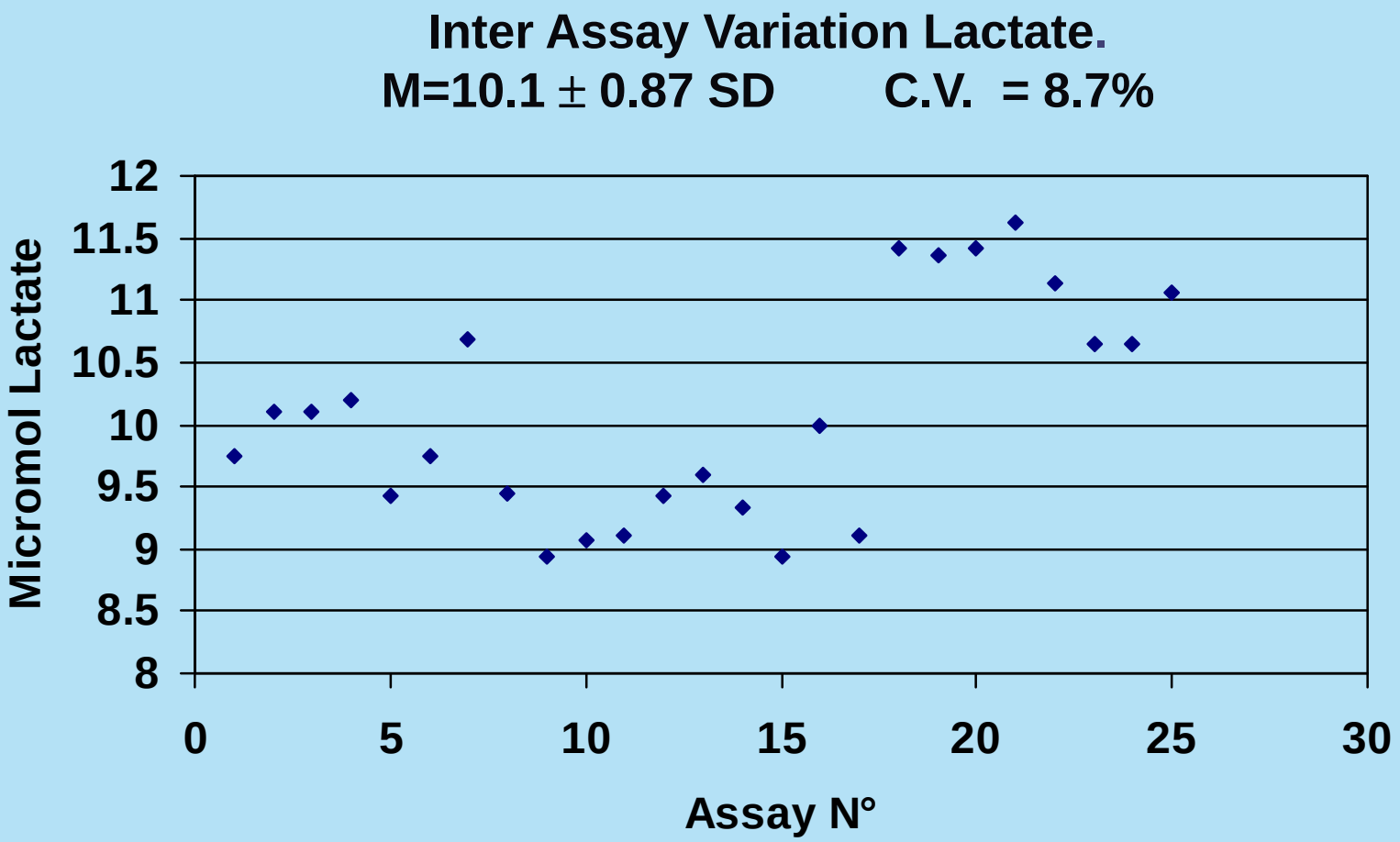
$$\text{Lactate} + \text{NAD}^+ \xrightarrow{\text{LDH}} \text{pyruvate} + \text{NADH} + \text{H}^+$$

Microfluorometric Assays

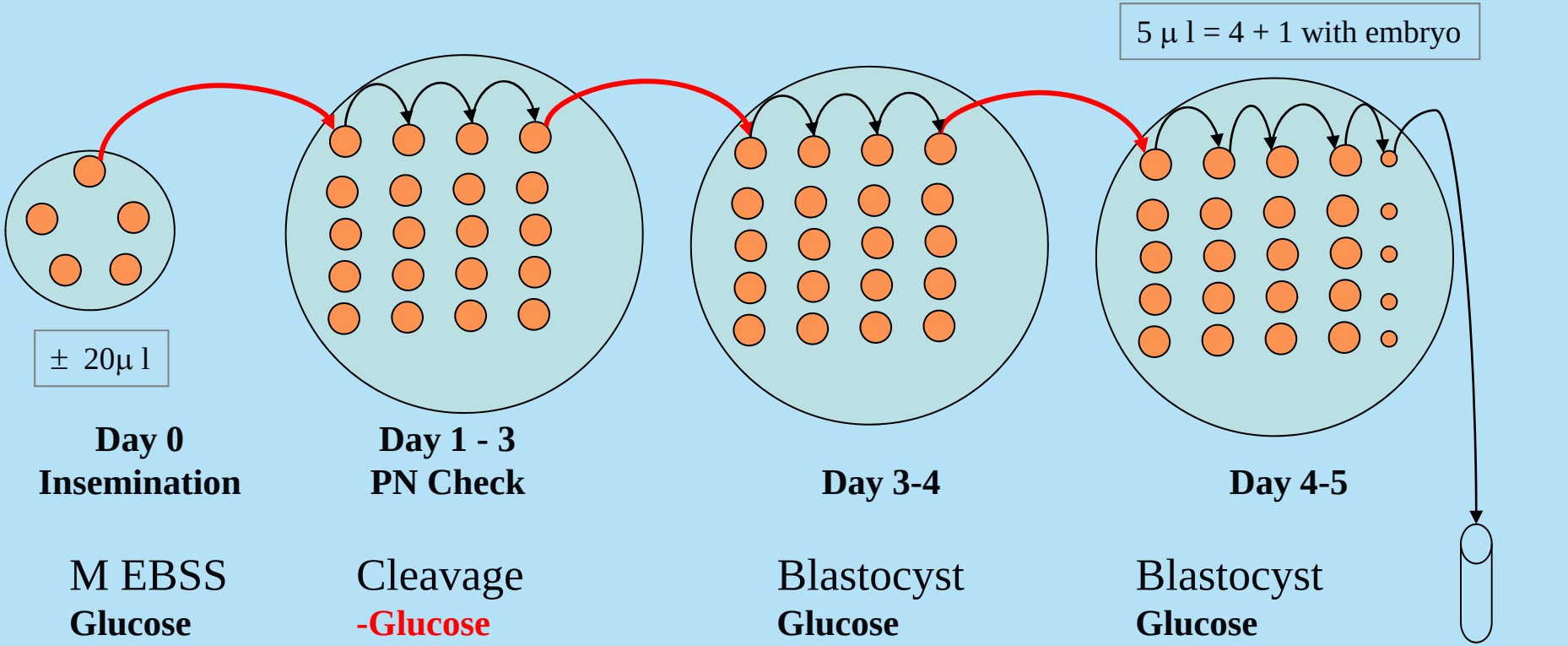
PRECLINICAL WORK



PRECLINICAL WORK



Sequential Culture System



Day 0 Insemination	Day 1 - 3 PN Check	Day 3-4	Day 4-5
M EBSS	Cleavage	Blastocyst	Blastocyst
Glucose	**-Glucose**	**Glucose**	**Glucose**
Lactate	**Lactate**	**- Lactate**	**- Lactate**
Pyruvate	**Pyruvate**	**Pyruvate**	**Pyruvate**
No A.Acids	**N.E. A.A.**	**N.E. + E. A.A.**	**N.E. + E. A.A.**

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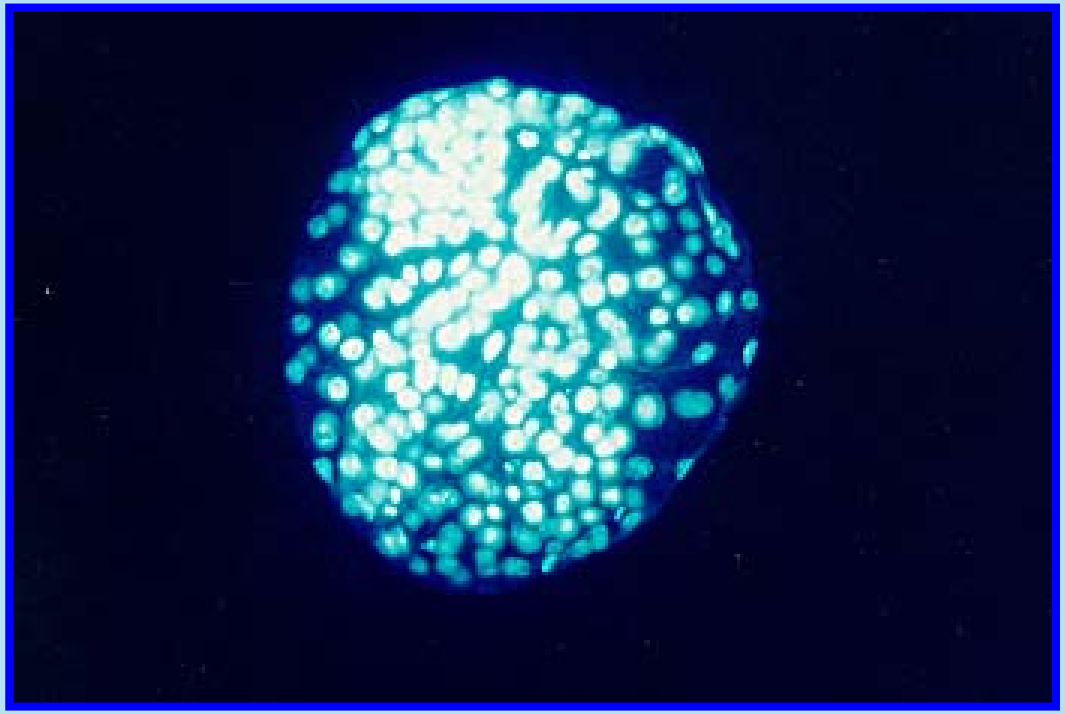
PRECLINICAL WORK

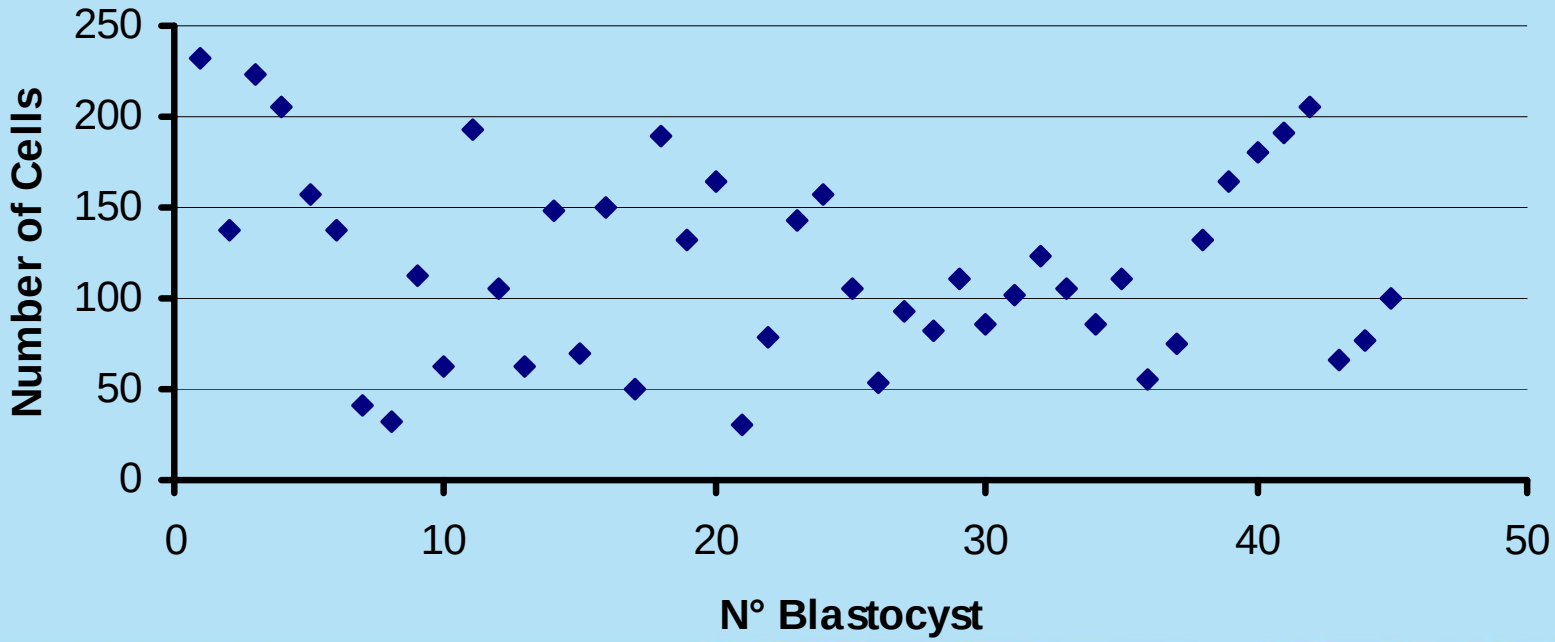
	N (%)
EMBRYOS CULTURED	**114**
BLASTOCYST OBTAINED ON DAY 6	**65/114 (57%)**
BLASTOCYST ASSESSED FOR TOTAL CELL COUNT	**45/ 65 (69%)**

BISBENZEMIDE STAIN



PRECLINICAL WORK

Total Cell Count in blastocysts
on day 6 of culture.
Mean = 118 S.D. = 53



Overall Results.

	1st CYCLE	2nd CYCLE	3rd CYCLE	4th CYCLE	TOTAL (%)
TRANSFERS	27	20	11	10	68 (92%)**
NO TRANSFER	0	2	3	1	6 (8%)**
UNKNOWN	1	0	1	2	4 (5.8%)**
BIOCHEMICAL PREGNANCY	3	2	0	2	7 (9.5%)**
CLINICAL PREAGNACY	9	7	5	2	23 (31%)**
MISCARRIAGE	2	2	0	0	4 (5.4%)**
DELIVERED	1	2	1	0	4 (5.4%)**
PREGNANCY RATE/ ET *	12/26 (46%)	11/18 (61%)	6/7 (86%)	2/7 (28%)	31/58 (53)%

Glucose-uptake and stage at D-5

STAGE	N	GLUCOSE pM/24h ± S.E.M.
HATCHING & EXPANDED BLASTOCYST	**77**	**524** ± 32
YOUNG BLASTOCYST	**62**	**536** ± 34
MORULA	**26**	**623** ± 64

Lactate-uptake and stage at D-5

STAGE	N	GLUCOSE pM/24h ± S.E.M.
HATCHING & EXPANDED BLASTOCYST	**77**	**524** ± 32
YOUNG BLASTOCYST	**62**	**536** ± 34
MORULA	**26**	**623** ± 64

Glycolytic Activity and stage D-5

STAGE	N	% GLYCOLYTIC ACTIVITY /24h ± S.E.M.
HATCHING & EXPANDED BLASTOCYST	**61**	**41** ± 3
YOUNG BLASTOCYST	**49**	**32** ± 4
MORULA	**12**	**46** ± 13

Glycolytic Avtivity
Pregnant Non-Pregnant

	PREGNANT N= 34	NON-PREGNANT N= 47	P
GLUCOSE UPTAKE pM/24h Mean ± S.E.M.	**626 ± 54**	**456 ± 50**	**0.02**
LACTATE PRODUCTION pM/24h Mean ± S.E.M.	**324 ± 32**	**336 ± 40**	**0.8**
% GLYCOLYTIC ACTIVITY Mean ± S.E.M.	**28 ± 3**	**51 ± 7**	**0.003**

CONCLUSION

Blastocysts in the group leading to a pregnancy h

- a higher Glucose-uptake
- a lower Glycolytic Activity

CONSIDERATION 1

DATA POLUTED BY TRANSFER
OF MORE THAN 1 EMBRYO.

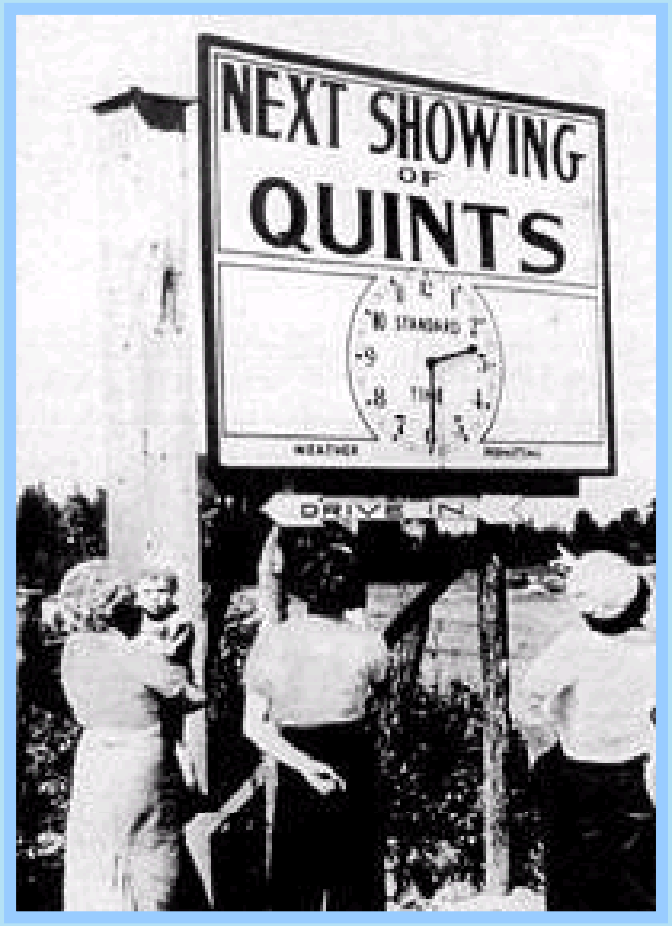
DIFFERENCE MAYBE MORE DISTINCT ?

CONSIDERATION 2

Practical aspects.

- Culture is labor intensive.
- Fluorimetric Assays fastidious.
- Absence of blastocysts with low activit

Dionne quintuplets



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