



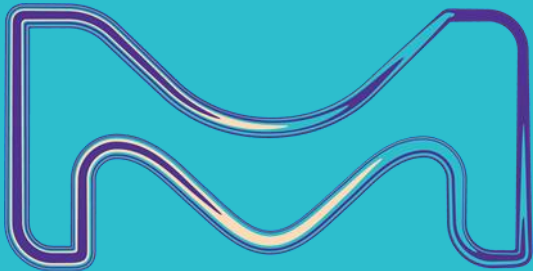
# In vitro potency assay for Follitropin alfa: a case study of worldwide (ongoing) registration

**CMC STRATEGY Forum Europe 2023**

**Session 2: Characterizing and Controlling Modes of Action**

Morgane Rochemont

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**MERCK**

# *In vitro* potency assay for Follitropin alfa

## Introduction

### Follitropin alfa

- Recombinant human follicle-stimulating hormone (r-hFSH, follitropin alfa) is a gonadotropin hormone.
- Starting dose 75IU, dose increase for treatment adjustment by 37.5IU.
- Registration status: approval in 1995 in EU; now registered in +100 countries.

### Purpose of the change

- Replacement of historical Steelman Pohley in vivo potency assay with a new in-house in vitro potency assay

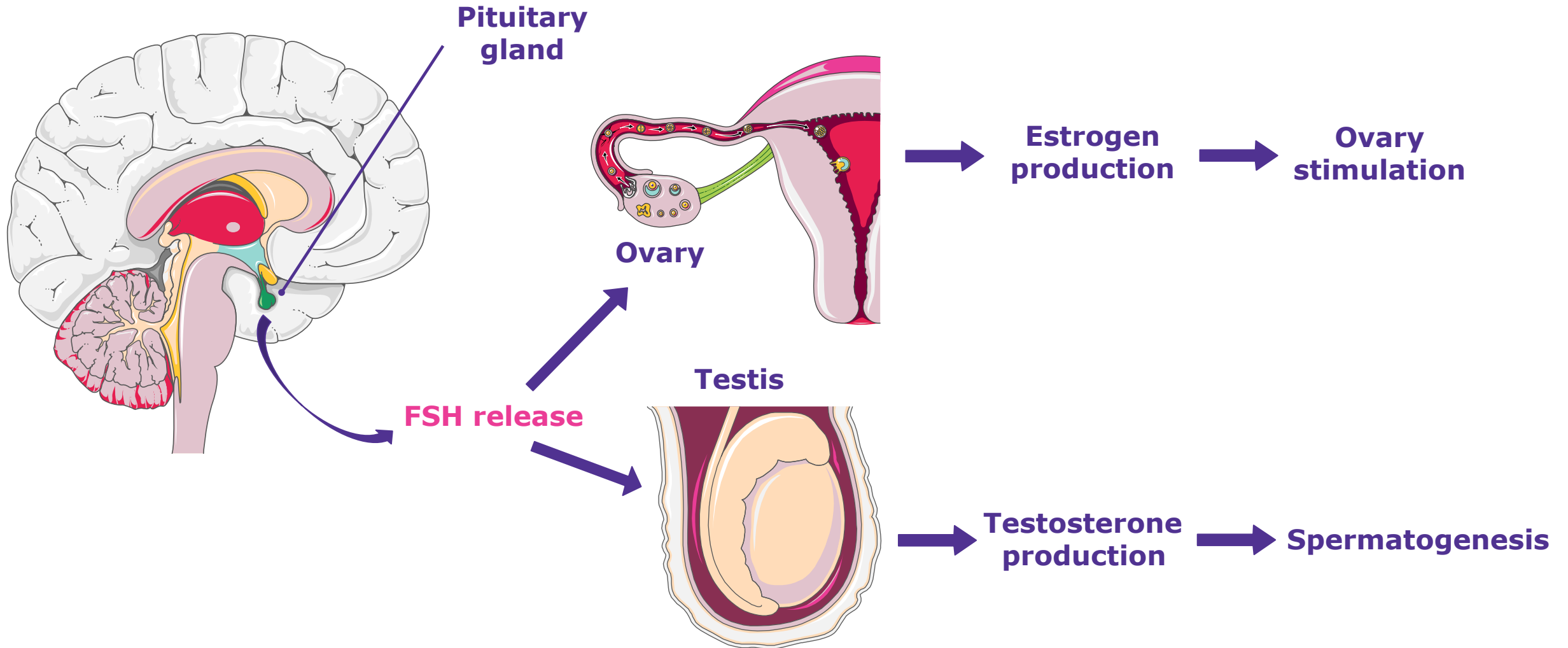
### Rational for the change

- 3R principles
- Improved method performance: precision, sensitivity
- Higher throughput



# *In vitro* potency assay for Follitropin alfa

## Mechanism of Action (1/3)

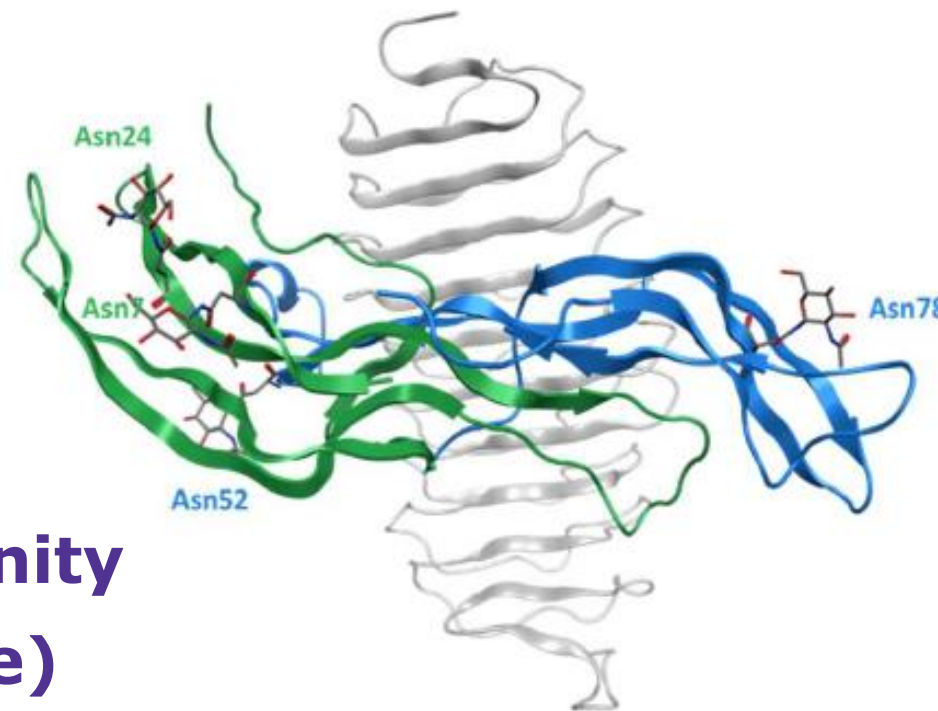


# In vitro potency assay for Follitropin alfa

## Mechanism of Action (2/3)



↑ **Sialylation**  
 -> ↓ **Binding affinity**  
 -> ↑ **PK (half-life)**



Site		
<b>α</b>	Asn52	Receptor recruitment Signal transduction
	Asn78	Close to the receptor activation area
<b>β</b>	Asn7	Metabolic clearance Serum half-life
	Asn24	Metabolic clearance Serum half-life

# In vitro potency assay for Follitropin alfa

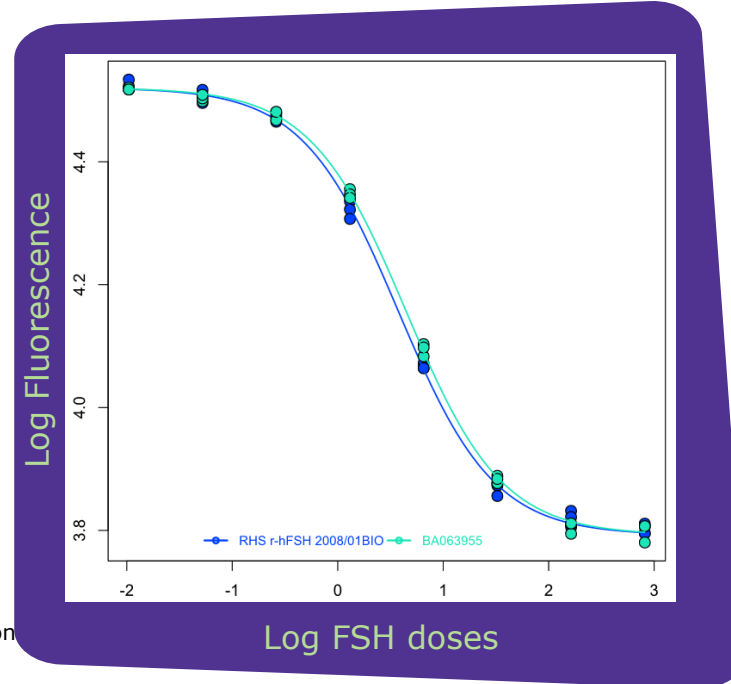
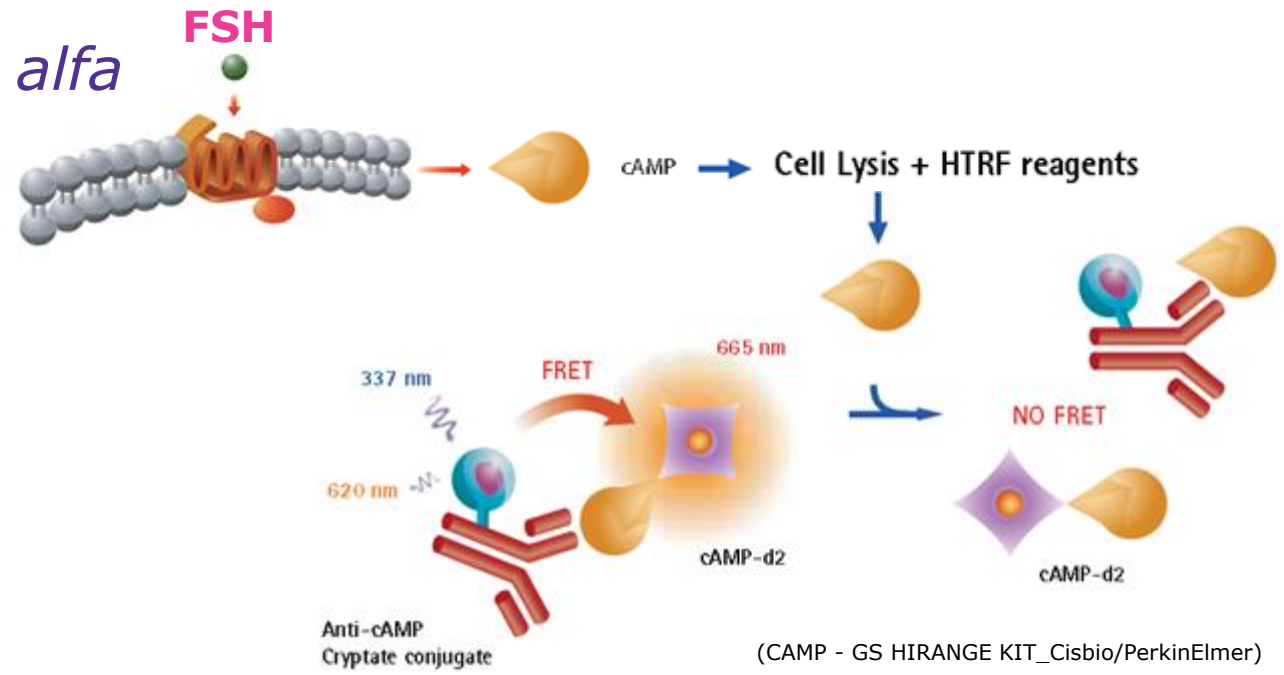
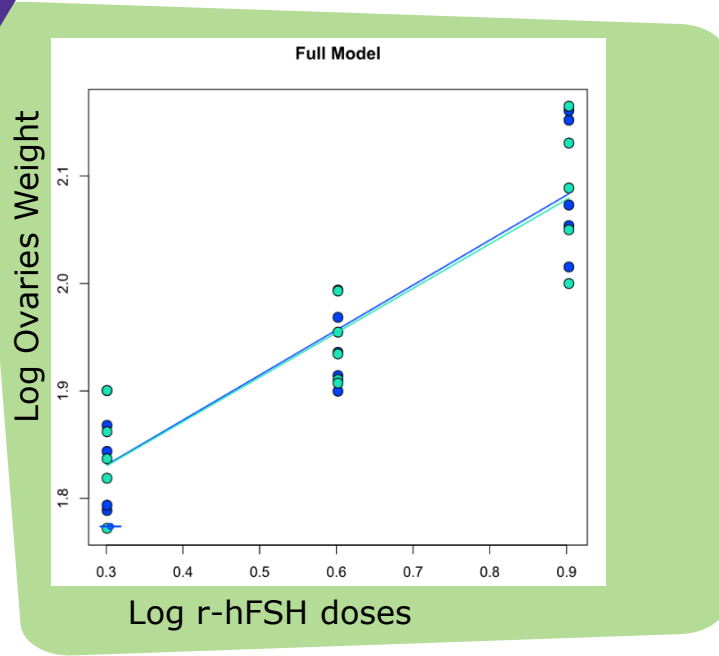
## Mechanism of Action (3/3)



**Sacrifice day 4 ovaries weight increase**

European Pharmacopoeia  
2285 and 2286

**In-vivo**



# *In vitro* potency assay for Follitropin alfa

## **In vitro bioassay Validation**



### **Linearity, Accuracy, Precision**

- 5 potency levels  
(RHS concentrations: 60%, 74%, 100%, 120%, 144%)
- 2 detection kits
- 2 FBS providers
- 2 operators



### **ICH Q2(R1)**

Validation of Analytical Procedures:  
Text and Methodology

### **suitability testing**

- Assay Suitability test
- Sample Suitability Test
- Minimal weight
- Combination of Assay Suitability Test

### **Range**

Range for which the method has demonstrated suitable level of Accuracy, Precision & Linearity

### **USP <1033>**

Biological Assay Validation

### **robustness**

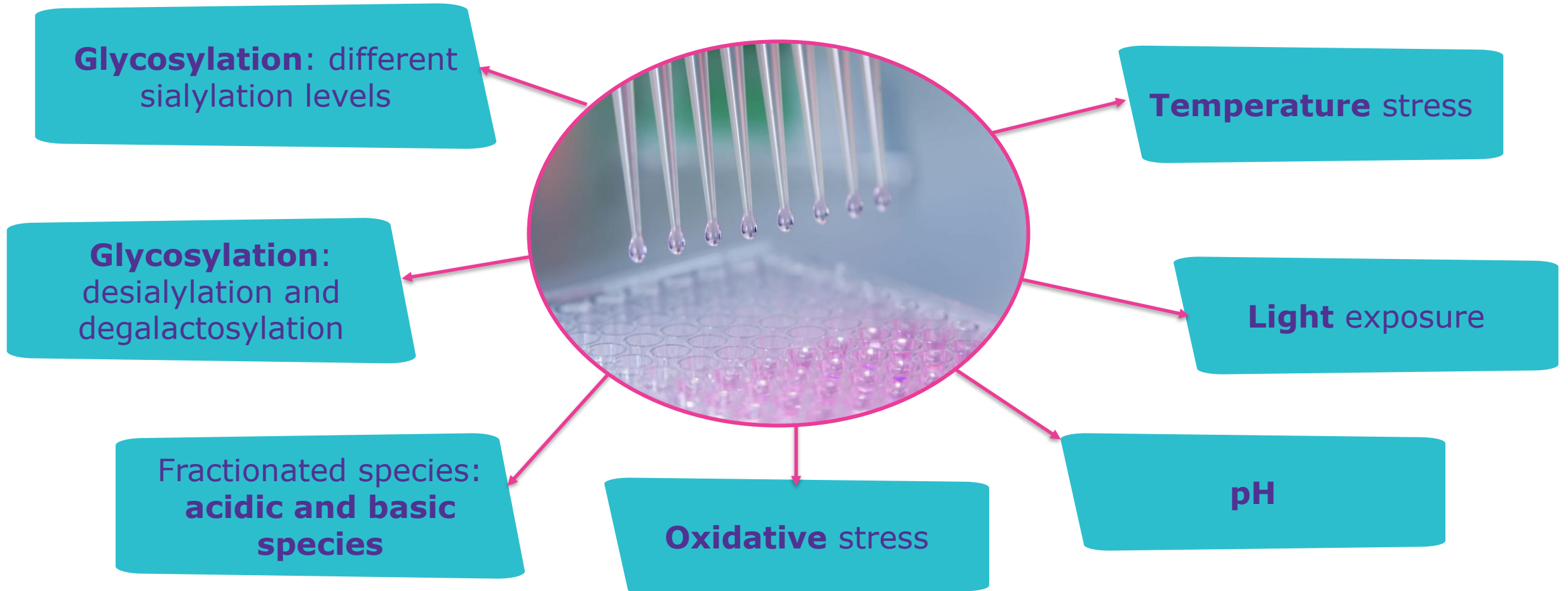
- Duration of cell stimulation with hormone
- Cell concentration
- Duration of incubation of the FRET plate

### **Specificity**

Other hormones (r-hLH, r-hCG, GH)

# *In vitro* potency assay for Follitropin alfa **In vivo – In vitro Comparability (1/4)**

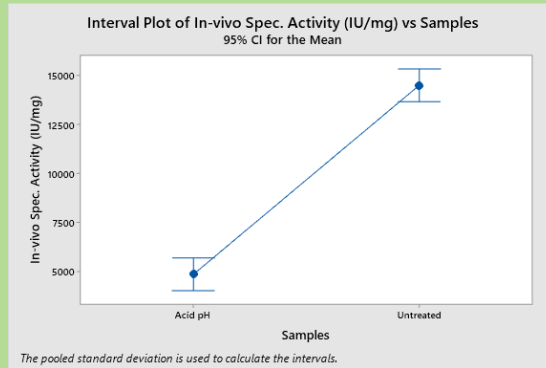
- Indirect comparison of results between both assays on stressed samples and variants
- Determination of correlation with other CQAs, and indirect comparison in vivo and in vitro



# In vitro potency assay for Follitropin alfa

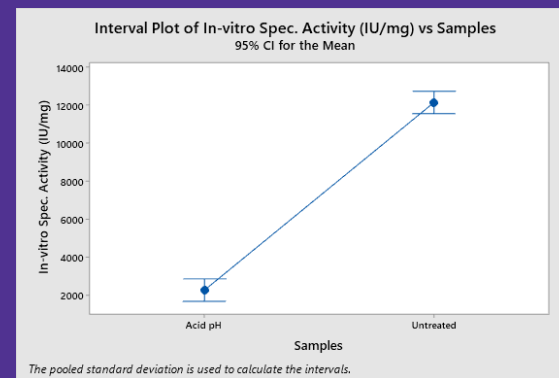
## In vivo – In vitro Comparability (2/4)

In-vivo



ANOVA test: p-value <0.001

In-vitro



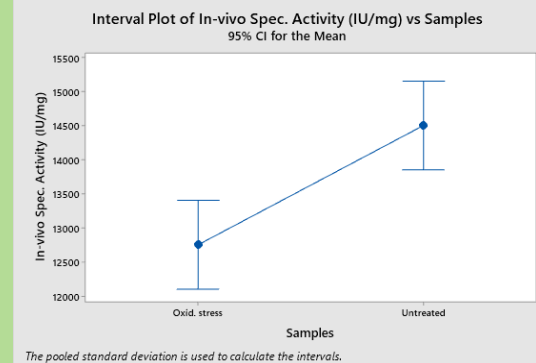
p-value <0.001

Dissociated subunits

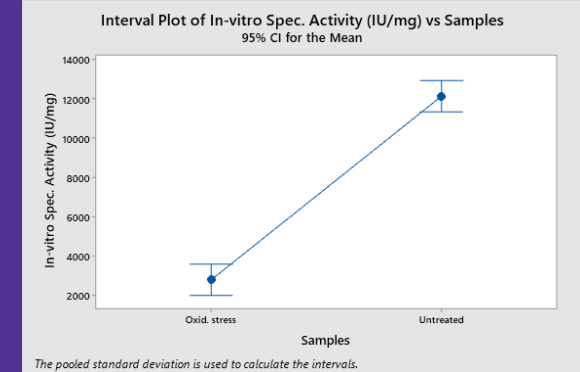
Both methods are sensitive

oxidation

Both sensitive, but in-vitro method is more sensitive



ANOVA test: p-value 0.006



p-value <0.001

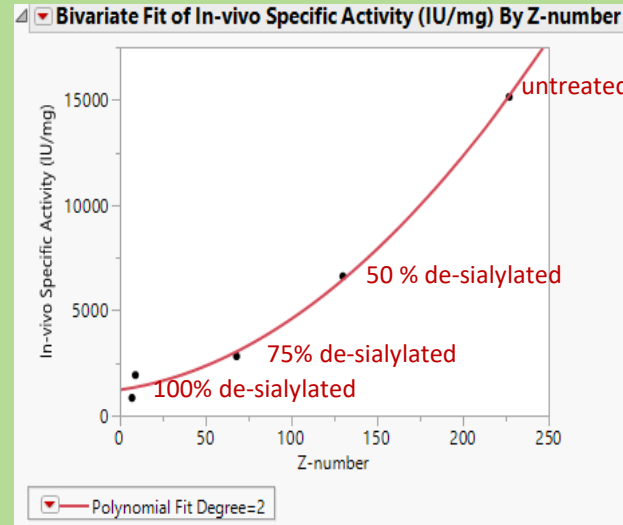


# In vitro potency assay for Follitropin alfa

## Comparability (3/4)

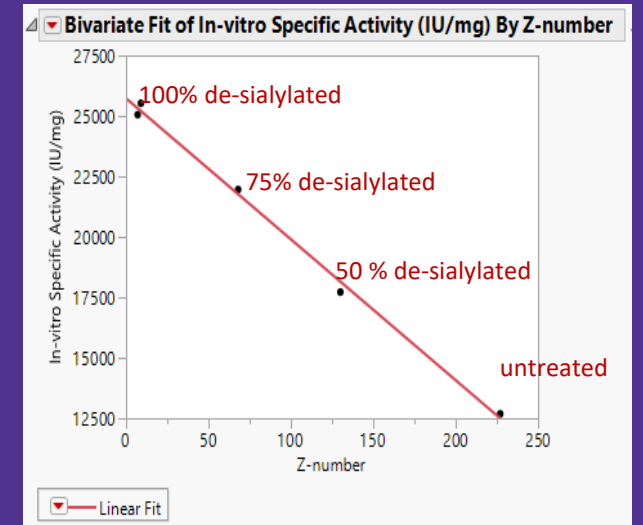
Sialylation (variants)  
Both sensitive,  
but opposite effect

In-vivo

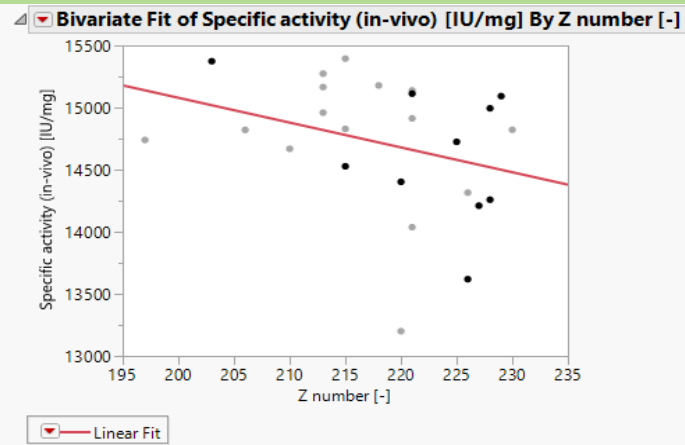


$R^2 = 0,995$

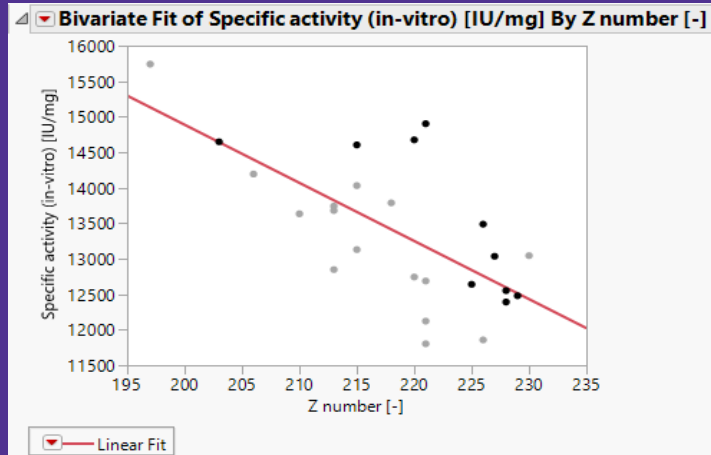
In-vitro



$R^2 = 0,996$



Significance of slope p-value **0.1262**



Significance of slope p-value **0.0002**

Sialylation (native samples)

In-vitro method is sensitive to slight sialylation differences while in-vivo method is not.

# *In vitro* potency assay for Follitropin alfa **Comparability (4/4)**

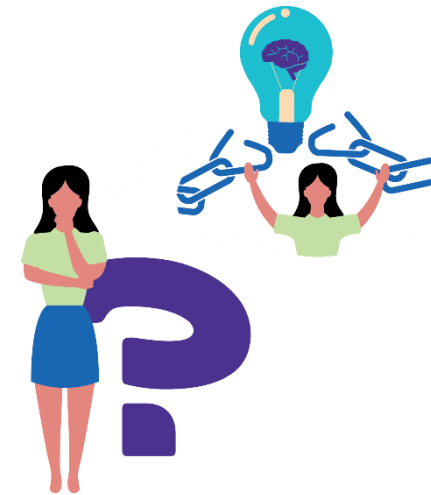
## Assays Performance

- Intermediate precision (estimated in vivo based on Reference standard retest vs IRS for extension of period of use):

In vivo: 8.7% vs In vitro: 6.4%

## Results

- Results not statistically comparable
- Dedicated specification for in vitro
- No impact on the drug product manufacturing process



## Reference Standard

- Recalibrated vs IRS with in vitro method > reference potency to be used for the determination of specific potency in QC of commercial batches.

# *In vitro* potency assay for Follitropin alfa

## Technical Challenges

### Sustainability of the assay (key reagents):

- Independency for critical reagents: detection kits

### Challenges for assay development:

- Design of a method able to mimic the MoA and to discriminate chemical and structural modifications that could occur to the molecule
- Identification of a read-out technology that could easily and precisely quantify an intracellular molecule



# *In vitro* potency assay for Follitropin alfa **Regulatory Challenges**

## **Comparability**

- Assays performance
- Assays results
- Switch from EP compendial test to in-house test



## **Key messages**

- Method was demonstrated to be suitable for Quality Control of FSH potency
- Integrated into the QC control strategy
- Integrating *in vitro* assay as alternative to *in vivo* assay in European Pharmacopeia would have a high impact on materializing 3R principles in QC of pharmaceutical products

# THANK YOU VERY MUCH !

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