

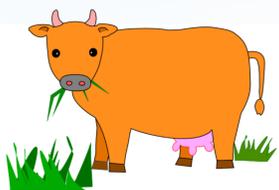
# Surface Plasmon Resonance Biosensor for Detecting Luteinizing Hormone

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## 1. Introduction

### Background

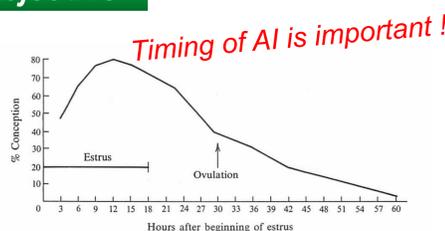


Livestock management depends on  
**Experience, Feeling, Luck**  
⇒ Low productivity

Applying engineering technique  
(Measurement of sexual hormones)

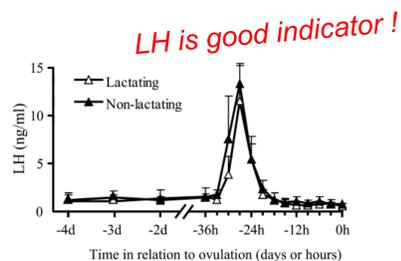
Highly efficient production

### Objective



The effect of time of insemination in relation to estrus on conception rate

G.W. Salisbury et al., *Physiology of reproduction and artificial insemination of cattle*, 2<sup>nd</sup> ed., 565-576, 1978



Temporal relationships between plasma concentration of LH from four days before to the time of ovulation

Endo et al., *J. Reprod. Dev.*, 58, 685-690, 2012

Develop a measurement system of LH that is easy to use for farmers

➔ **SPR biosensor**

## 2. SPR biosensor

### Apparatus

#### Sensor chip

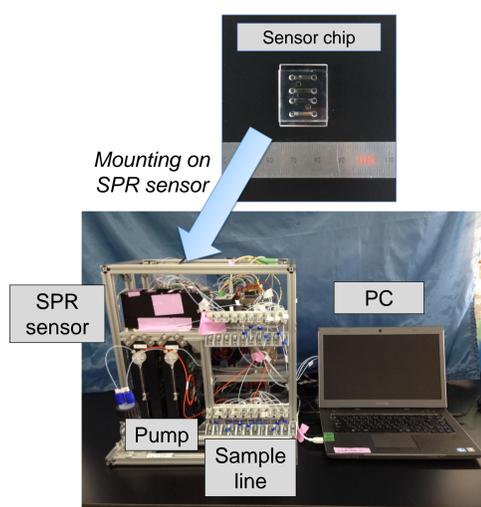
Sensor chip has 4 channels  
2 of 4 channels are connected to the SPR system

#### Sample line

10 samples can be connected to the SPR biosensor

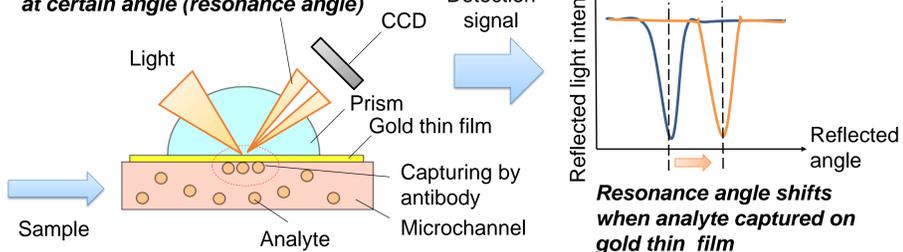
#### Operation

SPR biosensor is automated system  
It is controlled with PC



### Principle

Reduction of reflected light intensity at certain angle (resonance angle)



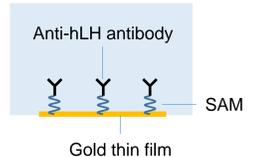
## 3. Measurement

### Procedure

Sandwich immunoassay was applied to amplify SPR signal

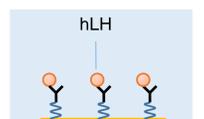
#### Step 1. Immobilization of primary antibody

Primary antibody (MyBioSource Inc., #MBS592103) was immobilized on gold thin film of sensor chip by amine coupling with carboxyl acid self-assembled monolayer



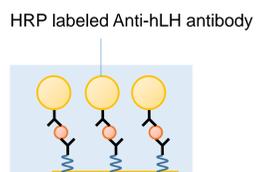
#### Step 2 Capturing luteinizing hormone (LH)

Human LH (Bio-Rad Laboratories, Inc., #PHP286) was used instead of bovine LH in this experiment



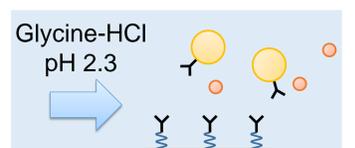
#### Step 3 Injection of HRP labeled secondary antibody

HRP was labeled to the antibody (Bio-Rad Laboratories, Inc., #MCA5806G) with HRP labeling kit (Dojindo Molecular Technologies, Inc., #LK11)



#### Step 4 Regeneration of primary antibody

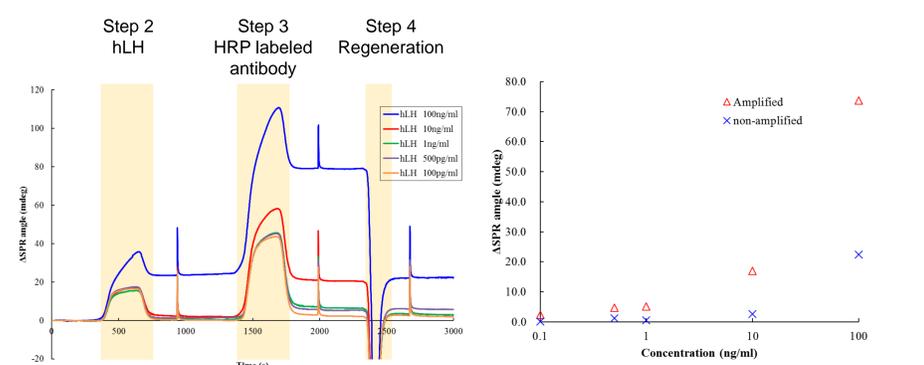
Glycine-HCl pH 2.3 was used for regeneration



#### Step 5 Repetition

Step 2 ~ 4 were repeated to measure different concentration

## 4. Results



SPR signal at each concentration

Shift of SPR angle of amplified (Red triangle) and non-amplified (Blue cross) at each concentration of LH (n = 2)

**SPR signal was amplified after HRP labeled antibody was injected**

**Shift of SPR angle of 500 pg/ml hLH can be detected by amplification**

## 5. Conclusion

- Developed SPR biosensor
- Succeeded detecting 500 pg/ml hLH by amplifying with HRP labeled secondary antibody