Biosensors for fertility and pregnancy in cattle

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Abstract

This project is focused on progesterone sensing, using both surface plasmon resonance (SPR) and lateral flow immunoassay (LFIA) methods with a new progesterone (P4) sensing material to develop cost effective assays for progesterone sensing in bovine serum and milk samples.

P4-PEG-OVA was synthesised, characterised and used for P4 detection. The P4-PEG-OVA sensor surface showed an improvement in surface response compared with two shorter ligand 4TP-P4-OVA and 4TPH-P4-OVA in SPR studies.

An analysis method has been developed and modified for bovine serum and milk analyses. The results indicated the P4-PEG-OVA ligand allowed sensitive P4 detection in SPR sensing and allowed bovine P4 cycle profiling. The SPR analysed data was compatible with the ECLIA and ELISA independent analyses and the P4 cycle of each of the three bovine milk samples showed a very similar trend and the extraction level was also consistent.

The P4-PEG-OVA ligand was used to develop a LFIA sensor strip, and the inhibition assay for bovine serum and milk analyses established. The results indicated that, after appropriate sample pre-treatment, the bovine estrous cycle profile could be detected. The LFIA method can be a potentially quick, easy and cost effective semi-quantitative P4 analysis for serum and milk samples.

A new material, polyhydroxyalkanoate (PHA) granules has been investigated for the possibility of developing a new surface biosensor. From the surface studies, the results indicated that the 3GNZZPhaC beads have the potential to become an alternative binding material for SPR sensing due to its unique gold binding property. A flow cell was designed, constructed, and tested on 3GNZZPhaC beads prior the preliminary SPR investigations.

The ZZPhaC beads also showed the gold binding property and ZZPhaC beads were used for SPR studies. The results suggested a possible application for them as a new SPR binding material for antibody detection.

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Abbreviations

4TP-P4	3-(pregn-4-ene-3,20-dione-4-yl)thiopropanoic acid
4TPH-P4	6-[3-[(pregn-4-ene-3,20-dione-4-yl)thiopropano-yl] amino]hexanoic acid
AFM	atomic force microscopy
Anti-P4	progesterone antibody
AuNPs	gold nanoparticles
BSA	bovine serum albumin
C	control line
CBG	corticosteroid binding globulin
CM5	carboxymethylate dextran
CL	corpus luteum
DCC	1,3-dicyclohexylcarbodiimide
DMF	dimethylformamide
DNA	deoxyribonucleic acid
dpm	disintegration per minute
EC20	the lowest concentration that can be distinguished from the background noise
EC50	the half maximal effect concentration
EC80	the highest concentration that can be distinguished from the background noise
ECLIA	electrochemiluminescence immunoassay
EDC	1-ethyl-3(-3-dimethylaminopropyl)carbodiimide
EDTA	ethylenediaminetetraacetic acid
EIAs	enzyme immunoassays
ELISA	enzyme-linked immunosorbent assay
FC1	flow cell one

FC2	flow cell two
FSH	follicular stimulating hormone
GC-MS	gas chromatography–mass spectrometry
GEPIs	genetically engineered polypeptides for inorganics
HBS-EP$^+$	SPR buffer contained 0.1 M HEPES, 1.5 M NaCl, 30 mM EDTA and 0.5% v/v Surfactant P20
hCG	gonadotropin
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPLC	high performance liquid chromatography
IFC	integrated micro fluidic cartridge
IgG	immunoglobulin G
kDa	kilodalton
LC-MS	liquid chromatography–mass spectrometry
LFDs	lateral flow devices
LFIA	lateral flow immunoassay
LH	luteinising hormones
LOD	limit of detection
NHS	N-hydroxysuccinimide
NZVP	New Zealand Veterinary Pathology Limited
P4	progesterone
P4-PEG	*N*-(13-(carbonylamino)-4,7,10-trioxatridecanyl)- 3-(pregn-4-ene-3,20-dione-4-yl)thiopropanamide
PHA	polyhydroxyalkanoate
PHB	polyhydroxybutyrate
PHBA	poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
pI	isoelectric point
pK	disassociate constant

OVA	ovalbumin
RIAs	radioimmunoassays
RU	response unit
SEM	scanning electron microscopy
SPR	surface plasmon resonance
SPRI	surface plasmon resonance imaging
T	test line
THF	tetrahydrofuran
TIR	total internal reflection
ZZ domain	antibody binding domain of protein A
θ_{SPR}	surface plasmon resonance angle
$\boldsymbol{\lambda_{max}}$	lambda(max)