



# **GR CALUX in illegal treatment detection: from targeted to untargeted screening strategy**

**7<sup>th</sup> BioDetectors**  
**2013**

***"DIOXINs/PCBs/POPs, ENDOCRINE  
DISRUPTERs (EDCs), OBESOGENS  
AND EMERGING POLLUTANTs"***



IZSTO is under the Ministry of Health

## Veterinary Public Health



Ministero della Salute

Our activities are focused on

**1. Control..** foodstuffs, feedingstuffs and live animals

**2. Detection..**

contaminants  
drug residues  
agents of zoonoses  
animal diseases

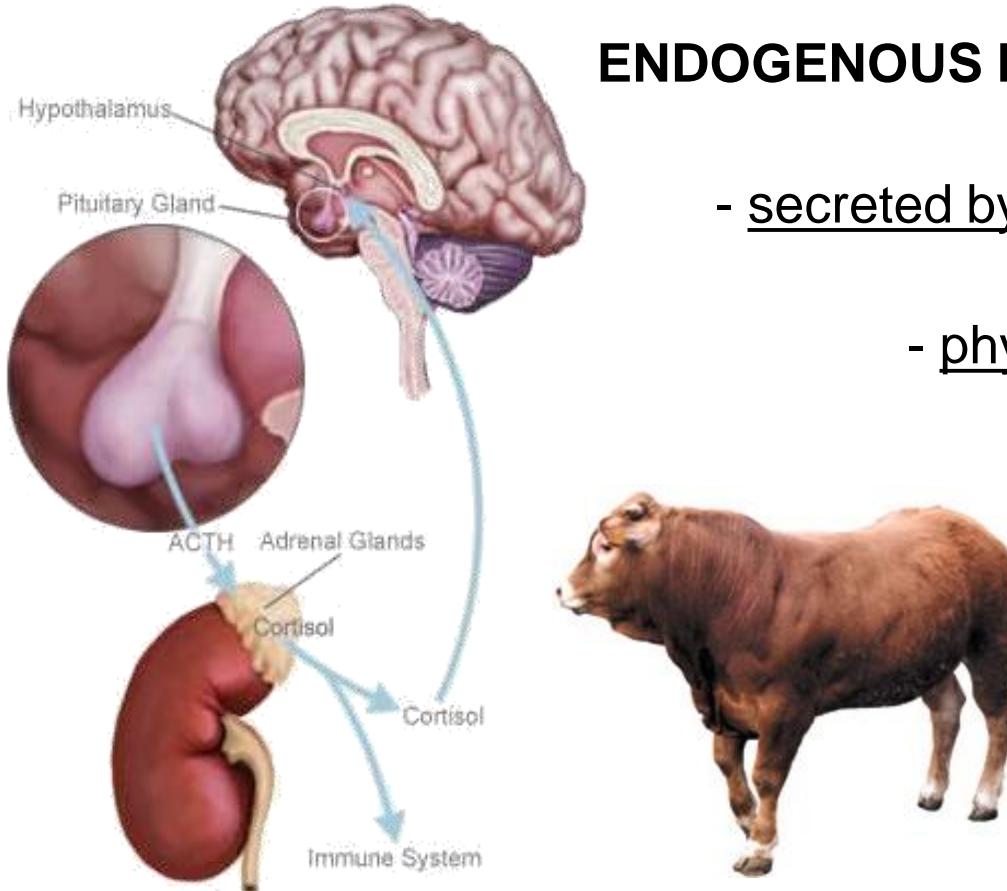
**3. Research..**





# Glucocorticoids

This group includes both natural and synthetic derivatives



**ENDOGENOUS hormones** (cortisol - cortisone)

- secreted by the adrenal cortex
- physiological activities
  - stress response
  - inflammation
  - immune function
  - hydroelectrolyte balance





# Glucocorticoids

This group includes both natural and synthetic derivatives

**SYNTHETIC molecules** (dexamethasone - betamethasone - flumethasone...)

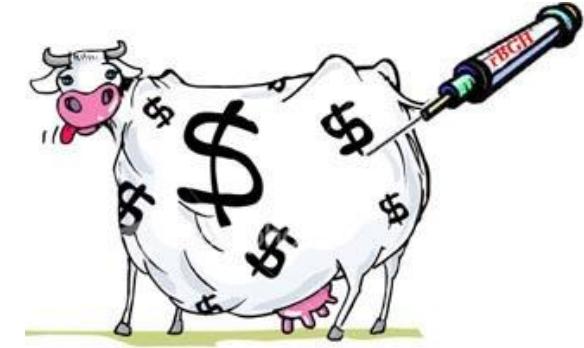
Therapeutic agents in veterinary practice; metabolic diseases, shock, inflammatory disorders

**They are also utilized illegally as growth promoters, either alone or in association with anabolic steroids**

**Our activity in live animals**

Correct use as therapeutic agents;  
respect of withdrawal time

Illicit treatment of producing animals





# Synthetic glucocorticoids

**Testing activity  
in live animals**

Urine samples

Screening analysis  
(ELISA)



Confirmatory analysis  
(LC-MS/MS)

**above 2 ppb**

**below 2 ppb**





## What's the matter with ELISA?

- Very susceptible to natural glucocorticoids (30% FALSE POSITIVE)

In Italy thousands of samples/year screened for steroids



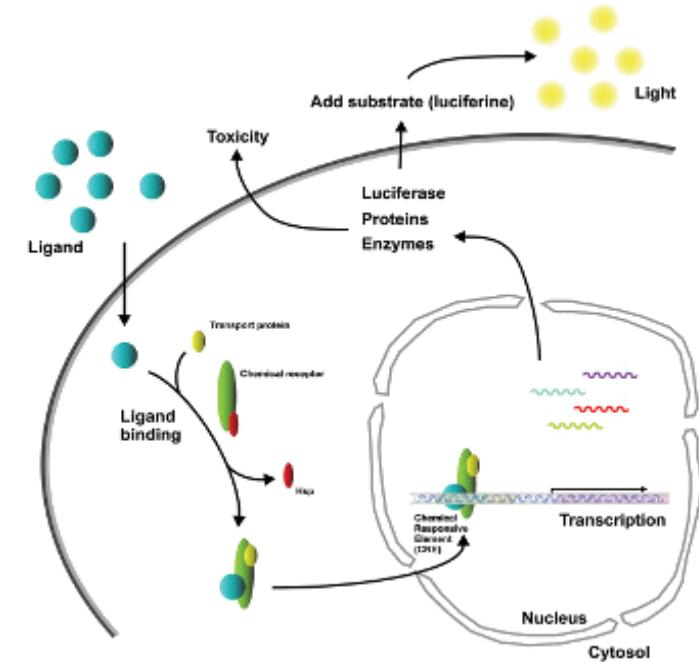
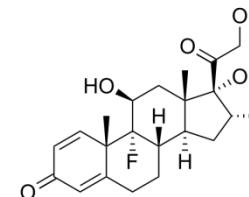
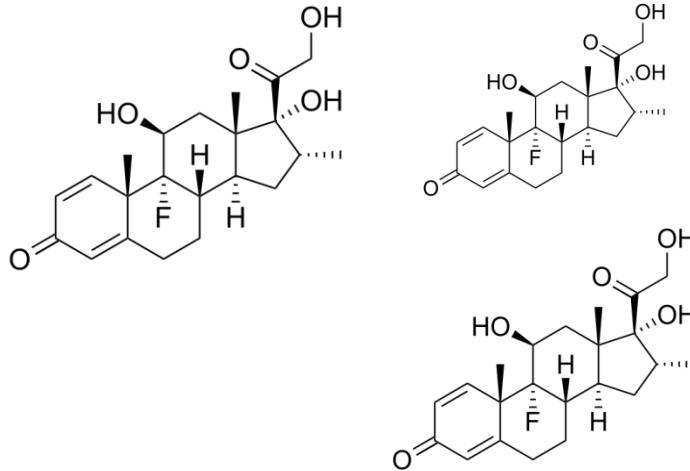
- It's limited to a short list of known molecules

Compound	% Cross-Reactivity
Dexamethasone	100
Dexamethasone 21 Acetate	162
Flumethasone	50
Betamethasone	43
Betamethasone 21 Acetate	73
Prednisolone	1.7



## ELISA vs GR CALUX

- Detection based on the glucocorticoid activity of each molecule, not on the “signature” of each molecule
- Potentially it could detect every molecule with glucocorticoid activity at a very low level
- Unimportant effect of natural glucocorticoids





## STUDY DESIGN

Evaluation of GR CALUX as screening tool for the detection of synthetic glucocorticoids in bovine urine

1. Activity of glucocorticoids on the method (REP - EC50)
2. Negative urine samples (from farm and abattoir)
3. Spiked samples
4. Incurred samples from experimental animals

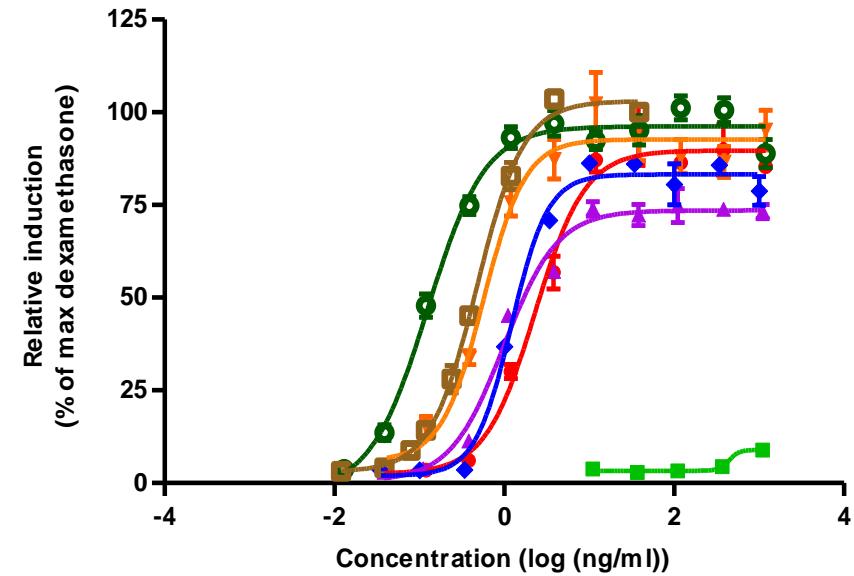
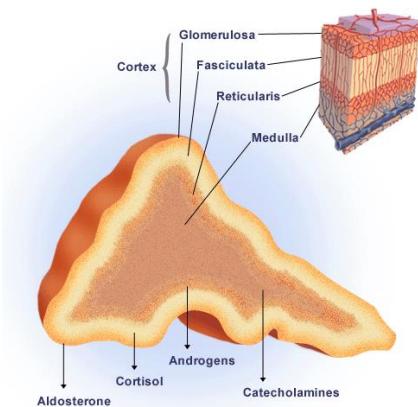




# 1. Pure compounds

## Synthetic molecules

Compounds	$EC_{50}$ (ng mL <sup>-1</sup> )	REP
flumethasone	0.13	3.60
dexamethasone	0.48	1.00
betamethasone	0.57	0.84
methyl-prednisolone	0.99	0.48
prednisolone	2.3	0.21
prednisone	-	-



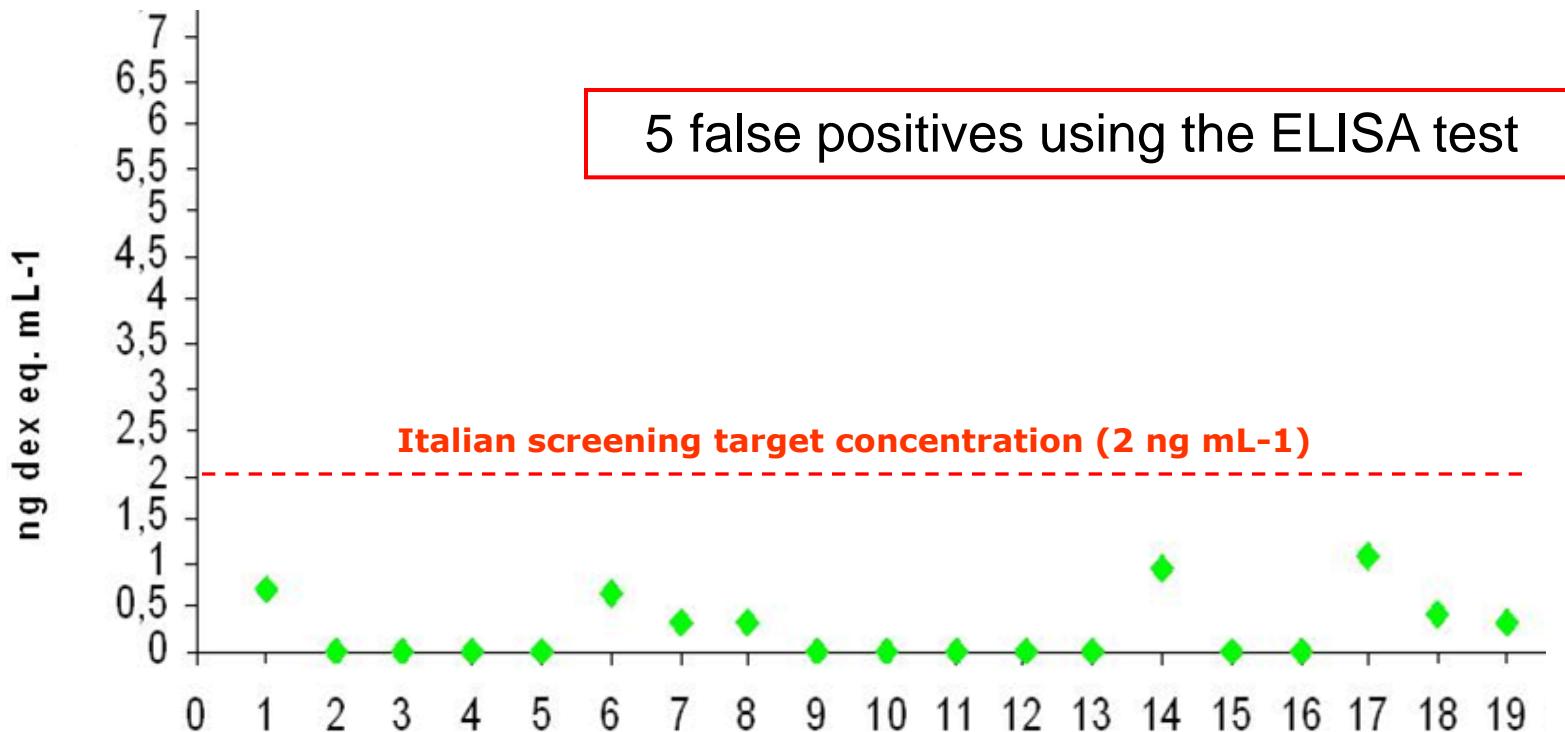
## Endogenous hormones

Compounds	$EC_{50}$ (ng mL <sup>-1</sup> )	REP
cortisol	5.71	0.084
cortisone	-	-



## 2. Negative urine from the farm

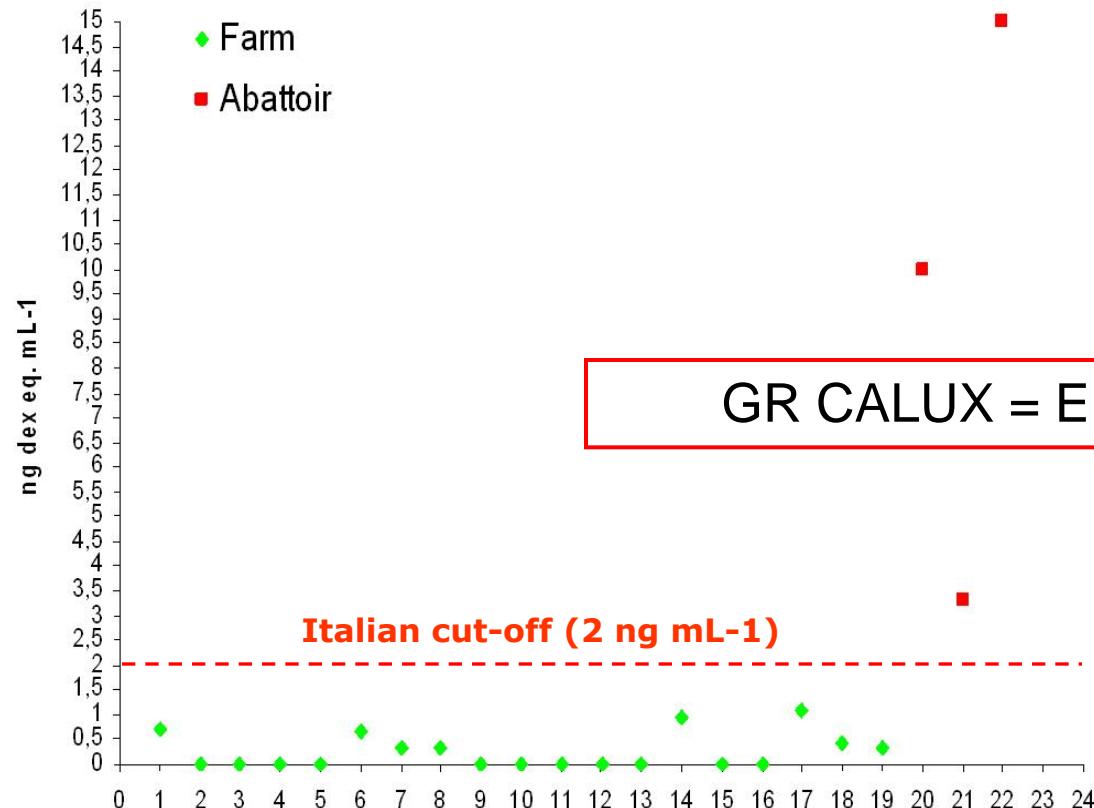
19 urine samples, calves bred under control conditions checked for natural corticoids by LC-MS/MS (total amount 1-8 ppb)





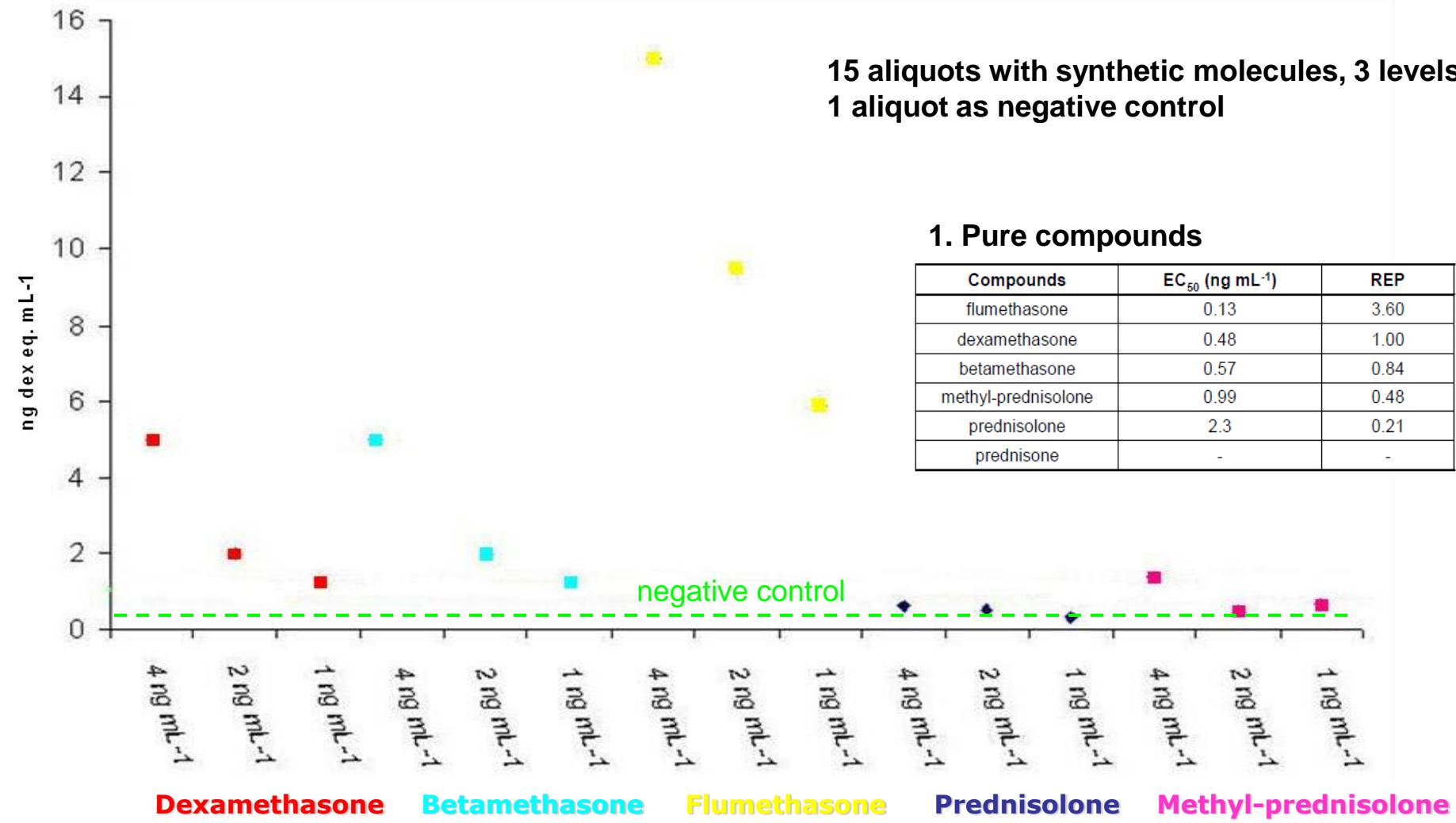
## 2. Negative urine from the abattoir

3 urine samples, calves bred under control conditions  
check for natural corticoids by LC-MS/MS (total amount **20-140 ppb**)





### 3. Spiked samples





## 4. Incurred samples

Urine samples LC-MS/MS tested	GR CALUX ng dex eq. mL <sup>-1</sup>
blank 1	0.60
blank 2	<LOQ (0.21)
blank 3	0.68
dexamethasone 0.1 ng mL <sup>-1</sup>	<LOQ (0.21)
dexamethasone 0.5 ng mL <sup>-1</sup>	0.56
dexamethasone 1 ng mL <sup>-1</sup>	1.90
dexamethasone 2 ng mL <sup>-1</sup>	2.27
dexamethasone 3 ng mL <sup>-1</sup>	5.02





## Conclusions

1. GR CALUX resulted as being very sensitive towards synthetic molecules
2. The physiological levels of endogenous GCs don't seem to influence CALUX measurements
3. At the slaughterhouse the increase in GCs levels could invalidate the analysis, the Italian Residues Control Plan prescribes the collection of samples at the farm
4. As expected prohormones can't be detected by GR CALUX (prednisone)
5. The technique looks a promising screening tool for the detection of illicit treatments in animals but more focused experiments are needed

**NEXT STEP..** *GR CALUX validation as screening test for GCs detection in bovine urine samples*

*Thank you*



## Acknowledgments

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