Influence of an estrogen treatment on the tissue specific expression pattern of estrogen receptors (ER):

Quantification of ER-alpha and ER-beta mRNA with real-time RT-PCR

Michael W. Pfaffl, Iris Lange, Andreas Daxenberger & Heinrich H.D. Meyer

Institute of Physiology, Research Centre for Milk and Food Weihenstephan, Technical University of Munich, Germany

INTRODUCTION

Goal of this study was to evaluate the deviating tissue sensitivities and the influence of the estrogen active preparation RALGRO on the tissue specific co-expression and regulation of both ER subtypes. RALGRO contains Zeranol (1x = 36 mg Zeranol), a derivative of the mycotoxin Zearalenon, which shows strong estrogenic and anabolic effects. It exhibits all symptoms of hyper-estrogenism in particular reproductive and developmental disorders. It is well known that steroids lead to an increased synthesis of specific proteins and it is proposed that estradiol can stimulate via ER-alpha its own receptor expression at least in the uterus. To quantify these possible transcripts also in low abundant tissues, sensitive and reliable real-time RT-PCR quantification methods were developed and validated on the LightCycler.

MATERIAL & METHODS

Eight heifers were treated over 8 weeks with multiple pellet implantations (0x, 1x, 3x, 10x) and after section Zeranol concentrations were measured by enzyme-immuno-assay (Zeranol EIA). In plasma and 4 representative edible tissues (longissimus dorsi, kidney, liver, peri-renal fat) concentration were elevated equivalent to the multiple treatment. In totally 15 different tissue ER expressions were quantified via real-time RT-PCR using the LightCycler. ER-alpha and ER-beta assay sensitivities were confirmed by detection limits down to 10 ssDNA molecules and linear quantification ranges between 10^2 to 10^9 molecules ($r>0.955$) with intra- and inter-assay variations of <19% to <30% respectively.

RESULTS

Both real-time RT-PCR were ER-alpha and ER-beta product specific and in all tissues both transcripts were found in different expression ratios (Table 1). To make the individual tissue expression pattern evident ER-alpha and ER-beta expression rates of 10 tissues were compared (Figure 1). High ERa/ERb ratios were examined in some muscle parts, liver, udder and uterus ; except in kidney and jejunum, the ERa/ERb ratios were <1.

With increasing Zeranol concentrations a significant down-regulation of ERa mRNA expression could be observed in jejunum ($p<0.001$) and kidney medulla ($p<0.05$) (Figure 2).

A positive high correlated ($r = +0.674$; $p<0.001$) co-expression of both ER subtypes was shown in uterus, liver, jejunum, abomasum, udder, spleen, lung, neck and hind leg muscularity.

DISCUSSION & CONCLUSION

In view of the data provided for sensitivity, linearity and reproducibility, the developed RT-PCR assay developed herein allows for the absolute and accurate quantification of ER-alpha and ER-beta mRNA molecules with a sufficiently high sensitivity even for tissues with low abundancies down to a few molecules. Our expression results indicate the existence of two ER subtypes in various bovine tissues, their different expression pattern and co-expression as well as their tissue specific regulation under estrogen treatment. These different expression patterns of ER-alpha and ER-beta could be regarded as support for the hypothesis that the ER subtype proteins may have different biological functions, especially in kidney and the jejunum where ER-beta expression ratio is vice versa in comparison to the other investigated tissues. In future more detailed study of ER-alpha and ER-beta must be investigated in all kidney cell types and in all parts of the gastrointestinal system to continue investigations of the ER regulation and its subtype physiological function.

Table 1: Expression pattern of ERa and ERb mRNA and expression ratio (ERa/ERb) in 25 ng bovine total RNA. Mean expression (in mRNA molecules) and variation coefficient (VQ) of 8 animals.

	ERalpha	VQ	ERbeta	VQ	ratio
uterus	980,000	53%	80,000	140%	12
udder	205,000	105%	7,250	64%	28
liver	200,000	87%	6,250	179%	32
lung	5,400	167%	900	94%	6
spleen	12,000	65%	10,400	104%	1.2
heart muscle	6,000	82%	4,200	56%	1.4
kidney medulla	10,200	52%	35,100	65%	0.3
kidney cortex	4,200	57%	29,300	51%	0.14
rumen	4,060	53%	1,350	109%	3
abomasum	4,600	84%	650	157%	7
jejunum	1,550	72%	2,150	152%	0.7
long. dorsi	79,400	61%	8,650	22%	9
hind leg m.	100,000	47%	5,850	76%	17
shoulder m.	60,500	83%	2,600	93%	23
neck muscles	145,000	79%	2,250	133%	64

Figure 1: Correlation of ERalpha and ERbeta co-expression in bovine tissues.

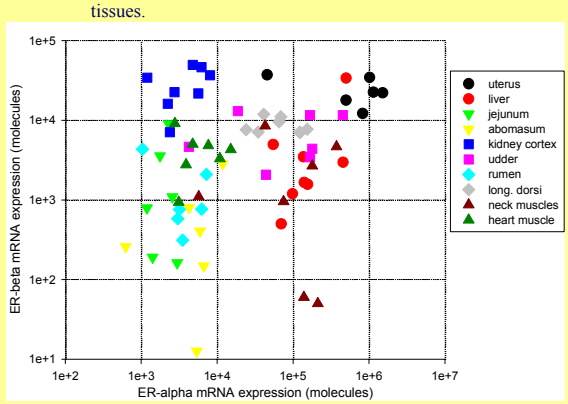

Figure 2: Relation between multiple RALGRO treatment with ERα expression.