Influence of Zeranol treatment on the tissue specific co-expression of estrogen receptors (ER) in various tissues: Quantification of ERα and ERβ mRNA with a real-time RT-PCR

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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, and 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 ERα 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 corresponding to the multiple treatment. In totally 15 different tissue ER expression profiles were quantified via real-time RT-PCR. ER α /ER β assay sensitivities and reliabilities 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

Real-time RT-PCRs were ER α and ER β product specific and in all tissues both transcripts were found in different expression ratios (Table 1). High ER α /ER β ratios were examined in some muscle parts, liver, udder and uterus; except in kidney and jejunum, the ER α /ER β ratios were <1. To make the individual tissue expression pattern evident all ER α and ER β expression rates were compared and shown with bi-directional error bars (Figure 1). Each tissue possesses an ER subtype specific expression pattern which stays relatively stable even under zeranol treatment and resulted in an ER α /ER β expression cluster.

With increasing Zeranol concentrations a significant down-regulation of ERα 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.

Table 1: ER α and ER β mRNA expression levels and expression ratio (ER α /ERβ) in 25 ng cattle total RNA. Mean expression (in mRNA molecules) and variation coefficient (VQ) of 8 animals.

	ER α	VQ	ER β	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

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 α and ER β 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 α and ER β 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 β expression ratio is vice versa in comparison to the other investigated tissues.

In future more detailed study of ER α and ER β 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.

Figure 1: ERα and ER β expression cluster in 15 bovine tissues.

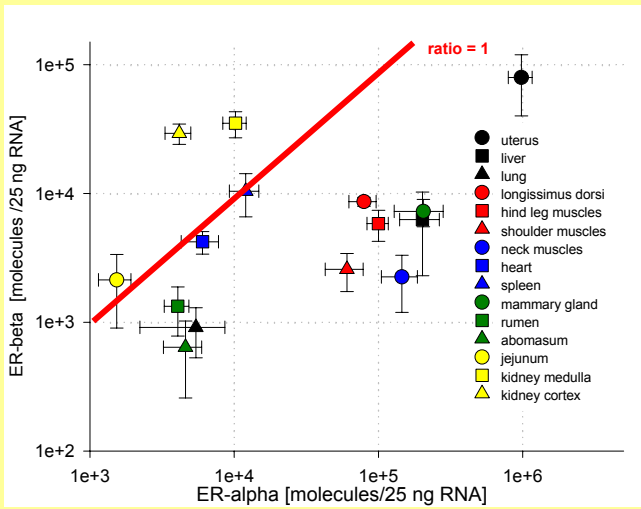

Figure 2: Relation between multiple RALGRO treatment and ERα expression.