High level iodine supplementation of the pregnant ewe alters IgG absorption and gene expression in the small intestine of the newborn lamb

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Background

- The survival of a newborn lamb dependent on the post natal transfer of colostral immunoglobulins into the blood stream
- Lambs are born without IgG as the placenta impedes immunoglobulin transfer to the foetus
- High level iodine supplementation of the pregnant ewe during late pregnancy, reduces the ability of the newborn lamb to absorb colostral immunoglobulin G (IgG; Boland et al., 2005a).
- Lamb appears to be pre-programmed in utero for this reduced absorptive capacity.
- Iodine is a key component of the thyroid hormones, thyroxine (T4) and triiodothyronine (T3).
- Evidence (Boland et al., 2008) suggests that failure of passive transfer (FPT) to the lamb may be mediated through alterations in circulating T3 levels at birth.

Introduction

- Main site of IgG absorption in the newborn lamb is at the lower ileum
- IgG absorption is believed to be mediated by using a receptor-mediated process called transcytosis (Rojas *et al.*, 2002).
- *Fc fragment of IgG receptor transporter Alpha (FCGRT)* and *polymeric immunoglobulin receptor (pIGR)* have been associated with post natal IgG absorption.
- However, the exact control of IgG transfer at cellular level is unclear

Hypothesis

- High level iodine supplementation of the ewe
  - alters thyroid hormone status of the new born lamb
  - and in turn alters gene expression in the small intestine
  - reducing the lambs ability to absorb maternal immunoglobulins

Materials and methods

- 30 twin bearing ewes were offered a basal diet of grass silage ad libitum plus 750 g/ewe/day of an 16.5% CP concentrate feed

Plus one of the following (n=15)

C: no supplmentary iodine

I: 27mg/ewe/day of iodine in the form of calcium iodate

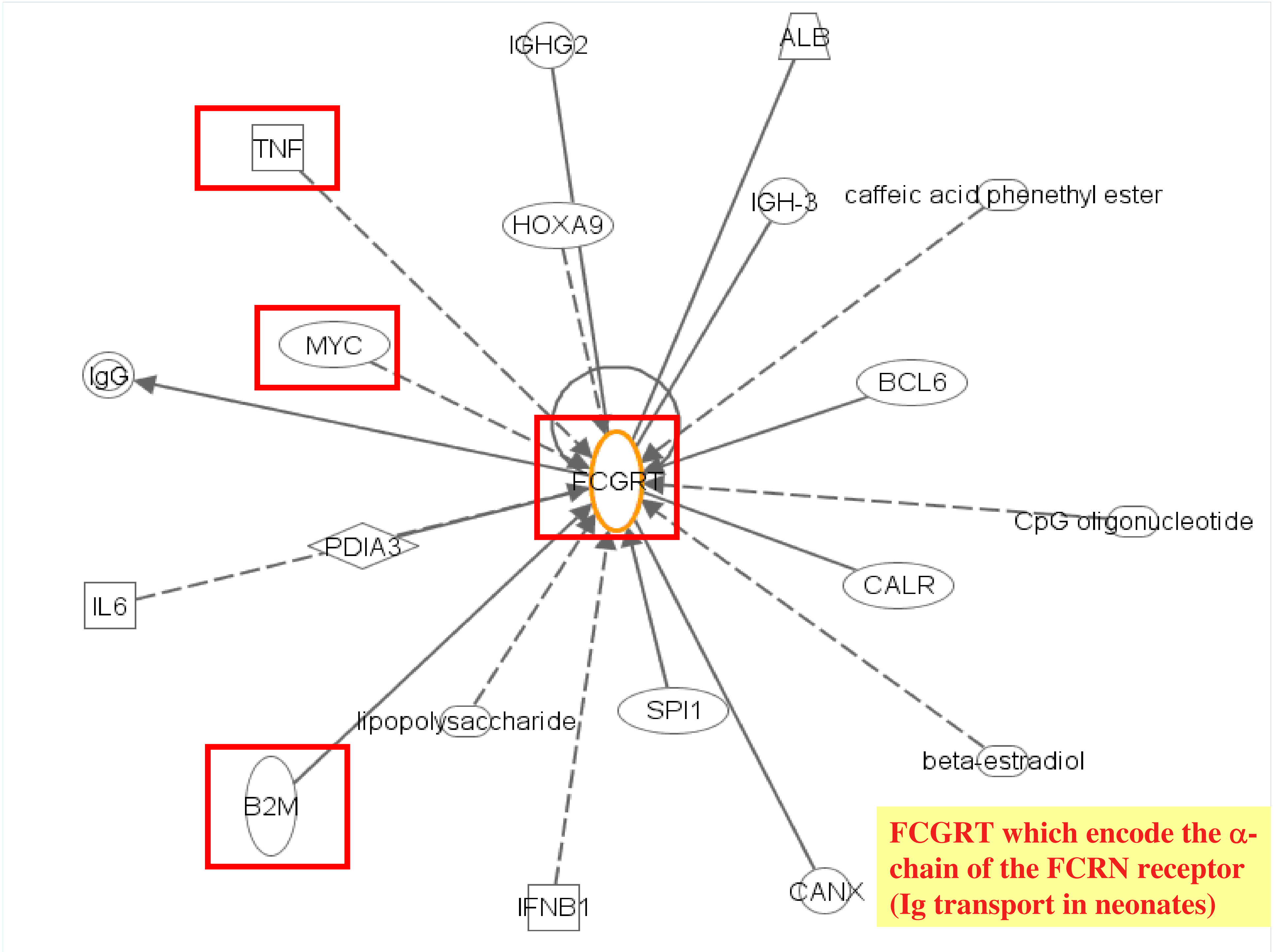
- Supplements were offered for the final 4 weeks of pregnancy

Materials and methods

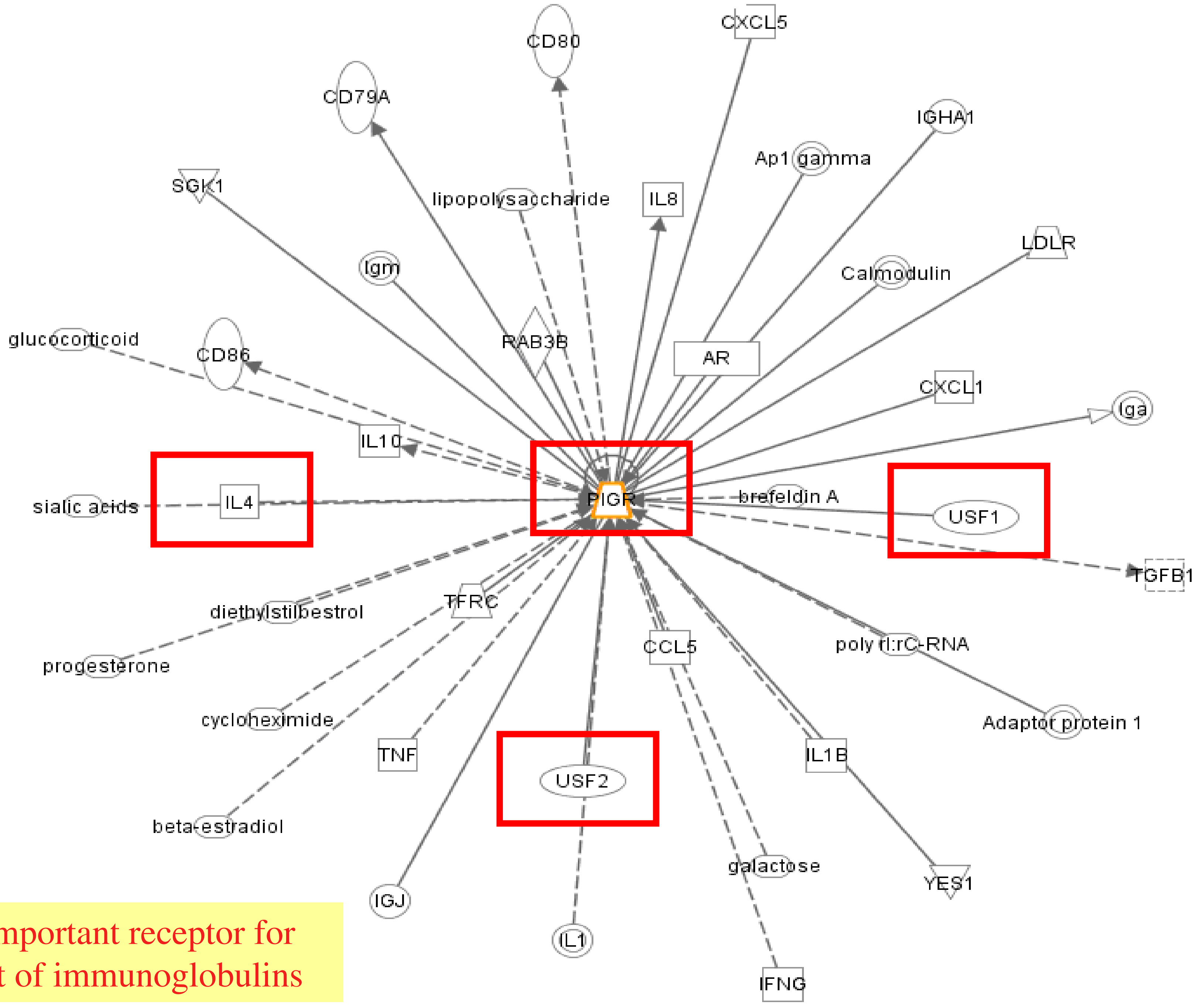
- 10 lambs per treatment were slaughtered immediately post partum
- Blood sampled prior to slaughter
- Ileal tissue harvested and stored at -80 C for gene expression studies
- Remaining lambs were fed known quantities of colostrum up to 18 h post partum
- At 24 h post partum their twin mate was blood sampled for serum IgG concentration

Gene identification

- Literature review and IPA (Ingenuity Pathway Analysis) 10 mammalian genes identified to be pivotal for the absorption of Ig's in the gastrointestinal tract of neonates



FCGRT which encode the α-chain of the FCRN receptor (Ig transport in neonates)



pIgR is an important receptor for the transport of immunoglobulins

Candidate gene list

Gene Name:	**Function:**
myelocytomatosis oncogen (MYC)	Transcription regulator of *pIgR*
tumour necrosis factor alpha (TNFα)	Regulates immune cells
β2-microglobulin (B2M)	Component of the antibody transporter (*FCGRT*)
Fc fragment of IgG receptor transporter Alpha (FCGRT)	Transporter of IgG
polymeric immunoglobulin receptor (pIGR)	Transporter of immunoglobulins
upstream stimulator factor 1 (USF1)	Transcriptional regulator of *pIgR*
upstream stimulator factor 2 (USF2)	Transcriptional regulator of *pIgR*
interleukin-4 (Il-4)	Cytokine that regulates immune cells
thyroid hormone receptor α (THRα)	Receptor for thyroid hormones (T4,T3)
thyroid hormone receptor β (THRβ)	Receptor for thyroid hormones (T4,T3)

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Materials and methods

- **RNA isolation:**
- **Primers were designed amplify specific fragments:**

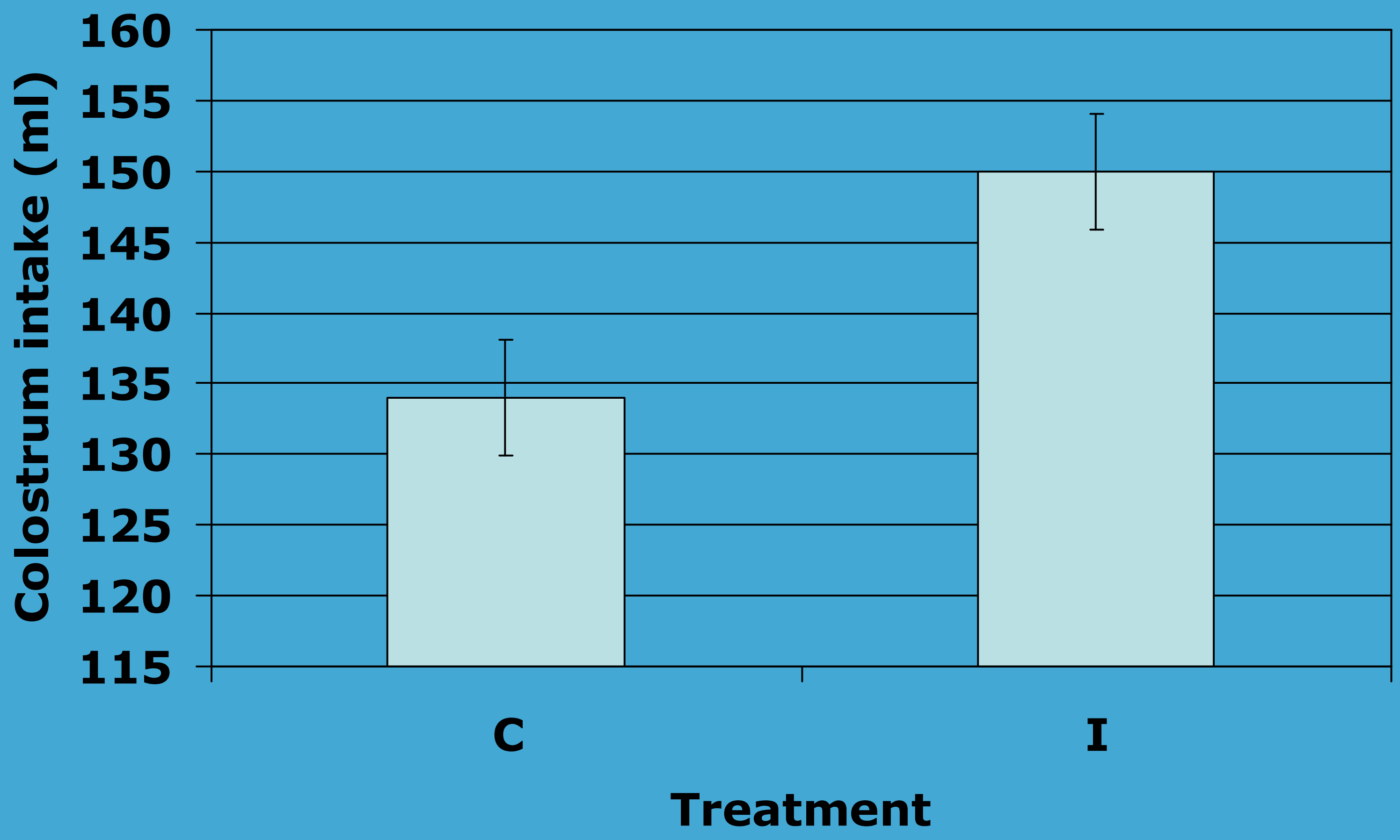
- Analysis of putative reference or 'housekeeping' gene were carried out using geNorm software
  - RPL19 selected as sole reference gene

- **$2^{-\Delta\Delta CT}$ method used to calculate changes in gene expression**

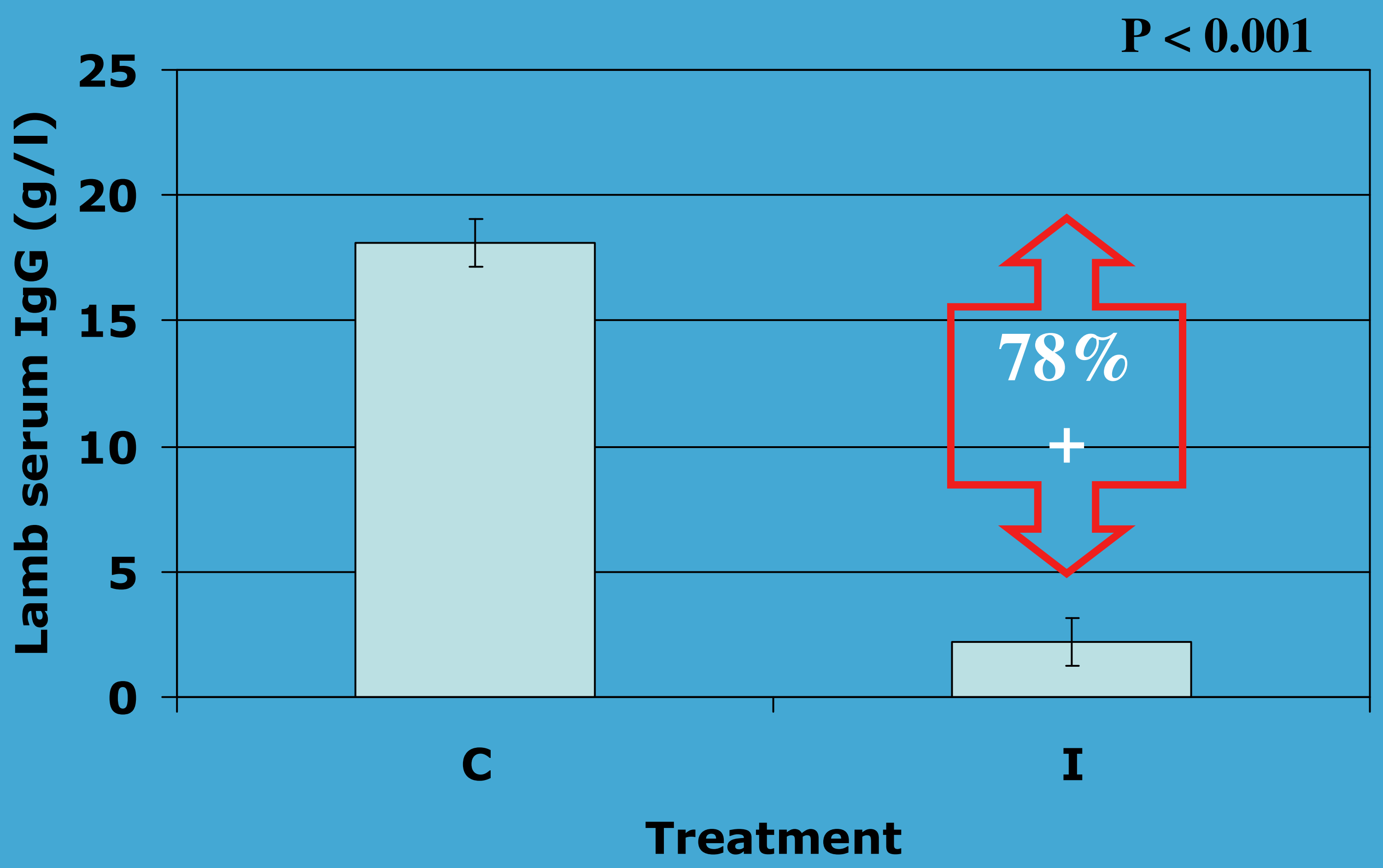
- Mean differences between the groups was tested using ANOVA (PROC MIXED).

Results

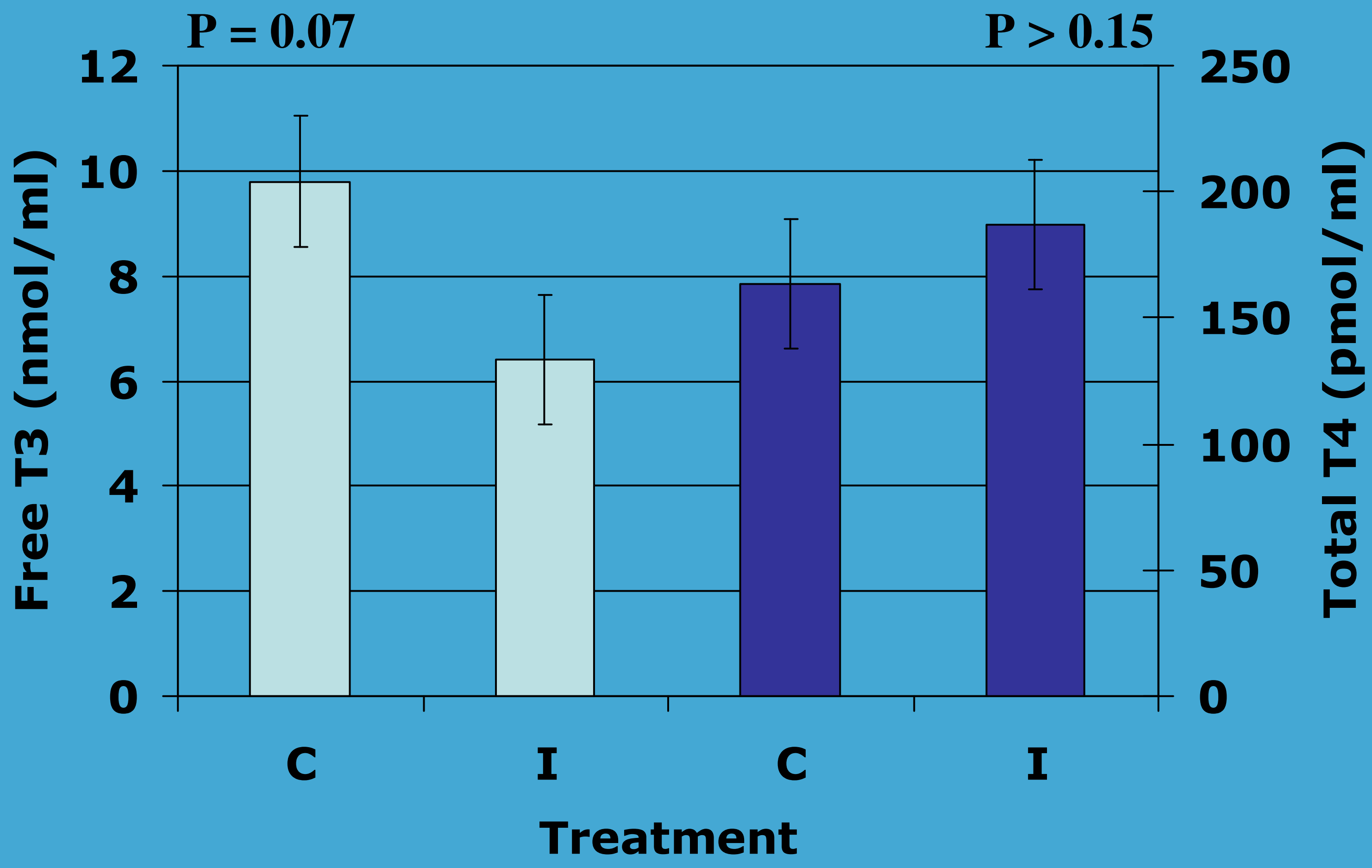
Effect of iodine supplementation on lamb colostrum intake up to 18 h post partum



Effect of iodine supplementation on lamb serum IgG concentration at 24 h post partum



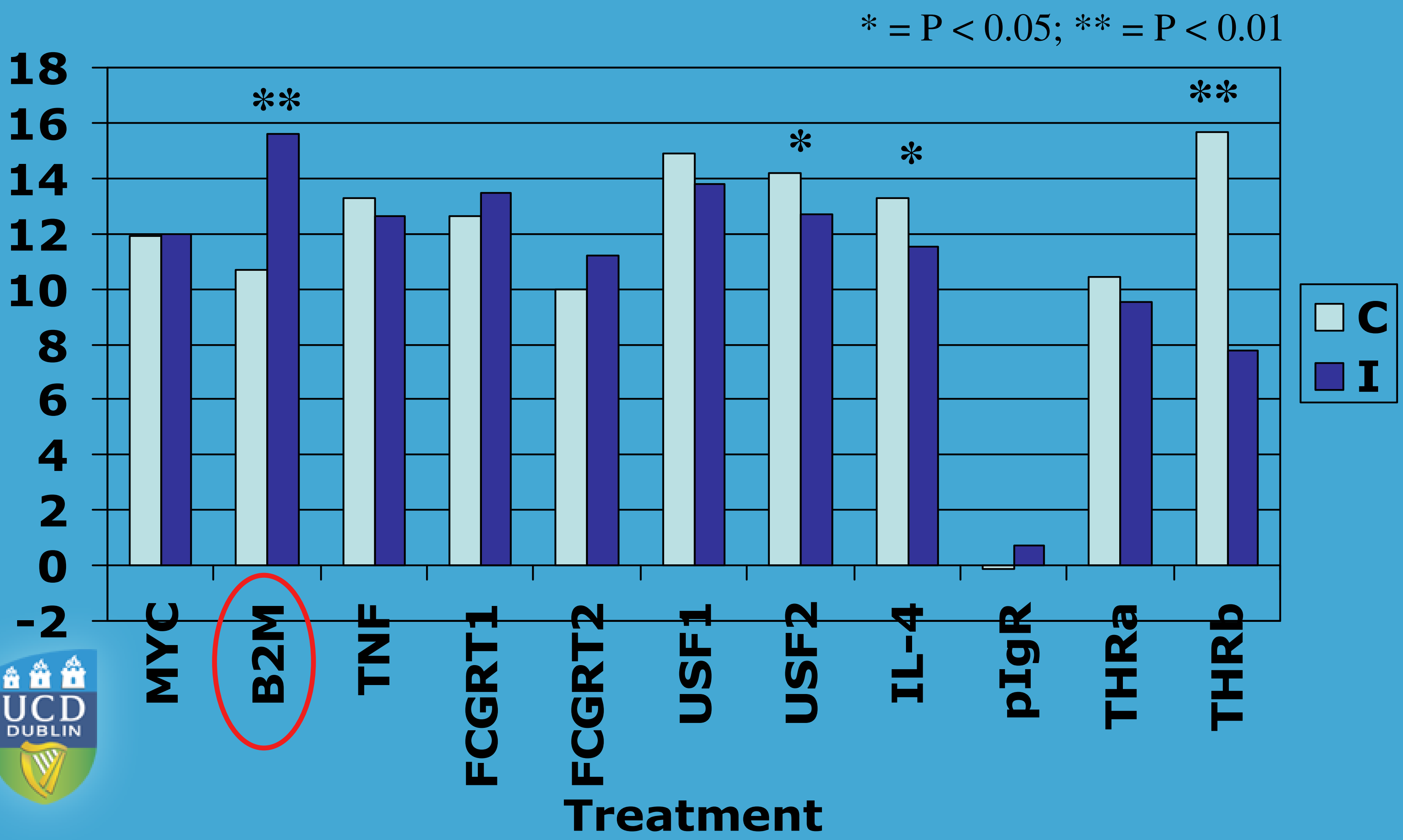
Effect of iodine supplementation on serum T3 and T4 concentrations at birth



Results

Gene expression

Expression profiles for all genes analysed



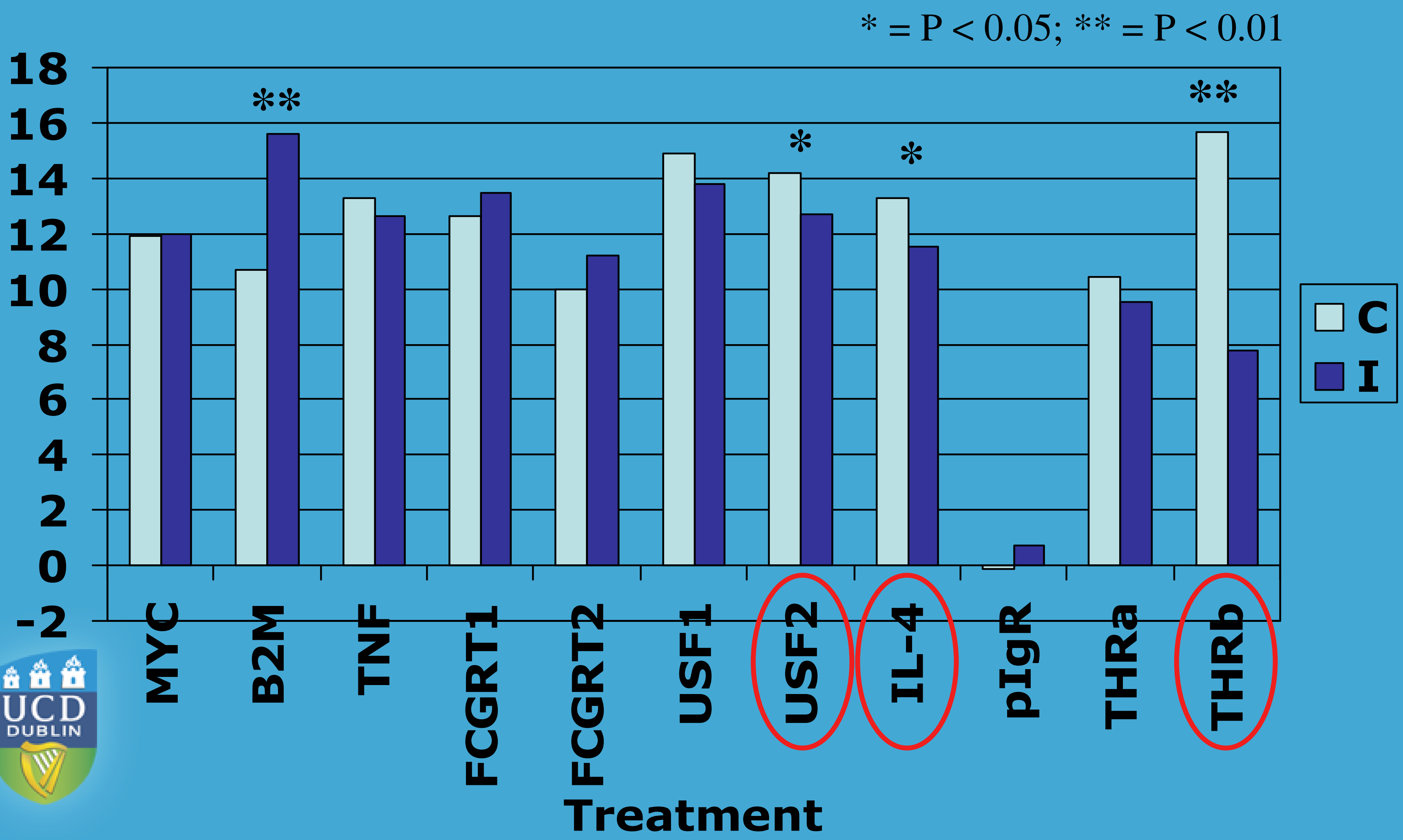
Up-regulated genes

- B2M ↑ (P < 0.01) in iodine supplemented treatment.

  Component of the antibody transporter (FCGRT)

  B2M has been shown to be involved with transport of IgG in neonates

Expression profiles for all genes analysed



Down-regulated genes

- USF2, IL-4 and THRβ↓ (P < 0.05) in the iodine supplemented treatment.

  USF2 is a positive regulator of the *pIgR*.

  IL-4 is a cytokine that regulates immune cells.

  THRb is involved in T3 mediated responses which include positive absorption of IgG in neonates

Summary

- Iodine supplementation of the pregnant ewe
  - Reduces free T_3 circulatory levels of her progeny at birth
  - Reduces lamb serum IgG concentration at 24 h post partum
  - Alters gene expression at the ileal level in the lamb at birth

Conclusion

- The well documented reductions in circulatory IgG levels of the lamb, following high level iodine supplementation of the pregnant ewe may be as a result of altered gene expression at the site of IgG absorption, probably mediated through changes in the thyroid hormone status immediately post partum and most probably in utero

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