Effects of conceptus sex and genetics on circulating thyroid hormones and IGFs in heifers at mid gestation depend on maternal genetic background

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Summary

Pregnancy is characterized by significant changes in maternal metabolism that are essential for adequate fetal growth. These changes are driven by conceptus demand and maternal supply and are also required for lactation. Here we use a purebred and reciprocal cross *Bos taurus taurus* (Bt, Angus) and *Bos taurus indicus* (Bi, Brahman) model to demonstrate significant effects of conceptus sex and genetics on maternal endocrine parameters at midgestation that manifest in a dam genetics dependent manner. In Bt and Bi heifers total thyroxine, but not triiodothyronine, concentrations were higher when carrying a male conceptus. However, free thyroxine was higher in Bt heifers carrying females, while Bi heifers showed a tendency for the opposite effect. In Bt heifers, insulin-like growth factor 1 and 2 were not affected by conceptus sex, but in Bi heifers IGF1 tended to be higher with a male conceptus. Furthermore, we found significant effects of conceptus genetics on total thyroxine and reverse triiodothyronine in Bi dams only. Apart from likely impact on heifer and conceptus growth, our data may help explain conflicting results reported for calf sex effects on milk production.

Keywords: cattle, maternal circulation, thyroid hormones, IGFs, conceptus sex, genetics

Introduction

There is growing evidence in human and animal models that conceptus sex influences maternal physiology (DiPietro *et al.* 2011, Mitchell *et al.* 2017, Petry *et al.* 2014, Retnakaran *et al.* 2015). These effects extend into and well beyond the postpartum period. In dairy cattle, accumulating data suggest conceptus sex/calf sex effects on lactation traits, although direction and magnitude of effects differ between studies. While an initial analysis by Hinde *et al.* (2014) concluded that gestation of a female conceptus leads to significantly higher yields for milk, fat and protein, subsequent analyses found either much smaller effects (Beavers & Van Doormaal 2014, Chegini *et al.* 2015, Hess *et al.* 2016), the opposite effect, (Græsbøll *et al.* 2015), or both, i.e., positive effects for female and male concepti, depending on parity/lactation number (Gillespie *et al.* 2017). Factors proposed to explain discrepant results include conceptus genetic effects. A biological basis for conceptus sex effects on lactation are fetal and placental hormones that may modulate maternal physiology and metabolism, including the mammary gland (Hinde et al. 2014). We have previously demonstrated that insulin-like hormone INSL3 enters the maternal circulation with significantly different concentrations in heifers pregnant with a male or female conceptus (Anand-Ivell et al. 2011) and conceptus sex affects serum metabolites in the peripartum dairy cow(Alberghina et al. 2015). Evidence for a role of conceptus genetics in physiology and metabolism of the mother is mounting but limited (Petry et al. 2007). Inter-individual variation in metabolic adaptation to pregnancy has long been recognized and factors that could account for this include maternal genetic factors (Prentice et al. 1995). However, general effects of maternal genetic background on physiological changes that occur during normal pregnancy remain unexplored.

Material and methods

Animals, experimental design and samples

All experiments were approved by the Animal Ethics Committee of the University of Adelaide (No. S-094-2005). We used *Bos t. taurus* (Bt, Angus) and *Bos t. indicus* (Bi, Brahman) dams to generate purebred and reciprocal cross concepti. Nulliparous Bt and Bi heifers aged 16-20 months were managed in one group on pasture supplemented with hay and went through standard estrous cycle synchronization procedures (Anand-Ivell *et al.* 2011). Pregnancies were established using 3 Bt and 2 Bi sires and confirmed by ultrasound scanning. Blood samples were collected from 72 pregnant dams upon sacrifice on Day153±1 post insemination where conceptus sex was confirmed. Plasma samples were frozen at -80^{o}C until further analyses. Temperature recordings were obtained from The Bureau of Meteorology, Australian Government.

Hormone assays

Concentrations of IGF1, IGF2 and total IGF binding proteins (tIGFBPs) were measured by RIA following separation of IGFs and IGFBPs by size-exclusion HPLC under acidic conditions as described previously (Sullivan *et al.* 2009). Total IGFBP obtained here is reflecting the total amount and binding affinity of IGFBP present in plasma. Total thyroxine (T_4), free thyroxine (fT_4), total triiodothyronine (T3) and free triiodothyronine (fT3) were assayed using coated tube radioimmunoassay IM 1447, IM 1363, IM 1699 and IM 1579 (Immunotech/Beckman Coulter, Prague, Czech Republic), respectively, according to the manufacturer's instructions. Reverse triiodothyronine (rT_3) was assayed in 100 ul plasma using reagents from double antibody radioimmunoassay kit BC 1115 (Biocode-Hycel, Liege, Belgium).

Statistical analyses

Effects of conceptus sex and genetics on maternal serum parameters were analyzed separately for each dam genetics to avoid confounding of fetal and maternal genetic effects. Data were analyzed with the linear model (GLM) procedure in SPSS (IBM® SPSS® Statistics 24, New York, United States) using the model

$$Y_{ik} = Intercept + F_j + S_k + F_j \times S_k + MW + MaxT + MinT + e_{ik}$$

where Y_{ik} is maternal parameter, F_j is fetal genetics effect, for Bt dams (j = BtxBt, BixBt) and for Bi dams (j = BixBi, BtxBi) with paternal genome given first, S_k is fetal sex (k = male, female), $F_j \times S_k$ is interaction between fetal genetics and fetal sex, MW is maternal weight at slaughter, MaxT is maximum temperature at day of slaughter, MinT is minimum temperature at day of slaughter, and e_{ik} is random error. We retained main factors fetal genetics and sex in the model irrespective of *P*-value, and interaction and covariates only when significant at $P < 0.05$, in the final models. Two-tailed *t*-test was used to compare differences between least square means and results were considered significant at $P < 0.05$.

Results and Discussion

At midgestation, at Day153 post insemination, we found significant effects of conceptus sex on plasma total thyroxine (T_4) in pregnant heifers. Dams with Bt genetics had 15% higher plasma T_4 concentrations ($P < 0.05$) when gestating a male conceptus and dams with Bi genetics ($P < 0.01$) a 22% higher T_4 concentration in comparison to Bt and Bi dams with female concepti, respectively. While free thyroxine (fT_4) concentration in plasma of Bi heifers followed a similar trend as T_4 concentration ($P < 0.10$), fT4 concentration in Bt heifers carrying a male conceptus was 9% lower ($P < 0.05$) as compared to those carrying a female. Neither total triiodothyronine (T_3) nor free T_3 or reverse T_3 (rT_3) were affected by conceptus sex.

The thyroid hormone axis and the insulin-like growth factor system are intrinsically linked in controlling growth, development and metabolism (Forhead & Fowden, 2014). We therefore analysed circulating IGF1 and IGF2 as well as IGF binding proteins (IGFBPs)and found that Bi heifers showed a tendency for higher IGF1 concentration ($P < 0.10$) when carrying a male conceptus, but no other IGF parameters were affected.

We next analysed the same maternal hormonal parameters for effects of conceptus genetics, i.e., in Bt heifers tested for differences between those carrying BtxBt and BixBt concepti, and in Bi heifers for differences between those with BixBi or BtxBi concepti. Plasma total T4 concentration in Bi dams with crossbred concepti was 25% lower ($P < 0.001$) than in those with purebred Bi concepti and the same dams also tended to have higher rT3 concentrations ($P < 0.10$), although total T3 and fT3 were not affected by conceptus genetics. Interestingly, no differences due to conceptus genetics were found in circulating hormones of Bt heifers.

In conclusion, our data indicate that the maternal thyroid hormone and IGF axes are significantly affected by conceptus sex and genetics in a maternal genetics dependent manner. Thyroid hormones and IGFs are intrinsically linked in growth and metabolism and significantly impact the mammary gland and lactation (Capuco et al. 2008, Etherton & Bauman 1998, Forhead & Fowden, 2014, Kim 2014). Therefore, the current data suggest that interactions of conceptus sex and genetics with maternal genetic and non-genetic factors impact fetal and dam growth. Furthermore, our data can help explain discrepancies in reported conceptus sex effects on lactation. Finally, from an evolutionary perspective, the modulation of key endocrine parameters of maternal physiology and metabolism by the conceptus demonstrates the complexity of interactions between conceptus and dam who are in genetic conflict to optimise pregnancy outcomes (Haig, 1993).

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