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Form of dietary selenium affects mRNA encoding interferon-stimulated and progesterone-induced genes in the bovine endometrium and conceptus length at maternal recognition of pregnancy

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Abstract

Widespread regions of the southeast United States have soils, and hence forages, deficient in selenium (**Se**), necessitating Se supplementation to grazing cattle for optimal immune function, growth, and fertility. We have reported that supplementation with an isomolar 1:1 mix (**MIX**) of inorganic (**ISe**) and organic (**OSe**) forms of Se increases early luteal phase (**LP**) concentrations of progesterone (**P4**) above that in cows on ISe or OSe alone. Increased early LP P4 advances embryonic development. Our objective was to determine the effects of the form of Se on the development of the bovine conceptus and the endometrium using targeted real-time PCR (**qPCR)** on day 17 of gestation, the time of maternal recognition of pregnancy (**MRP**). Angus-cross yearling heifers underwent 45-d Se-depletion then repletion periods, then at least 90 d of supplementation (**TRT**) with 35 ppm Se per day as either ISe (n = 10) or MIX (n = 10). Heifers were inseminated to a single sire after detected estrus (day 0). On day 17 of gestation, caruncular (**CAR**) and intercaruncular (**ICAR**) endometrial samples and the developing conceptus were recovered from pregnant heifers (ISe, n = 6 and MIX, n = 6). qPCR was performed to determine the relative abundance of targeted transcripts in CAR and ICAR samples, with the expression data subjected to one-way ANOVA to determine TRT effects. The effect of TRT on conceptus development was analyzed using a one-tailed Student's t-test. When compared with ISe-treated heifers, MIX heifers had decreased ($P < 0.05$) abundance of several P4-induced and interferon-stimulated mRNA transcripts, including *IFIT3*, *ISG15*, *MX1*, *OAS2*, *RSAD2*, *DGAT2*, *FGF2* in CAR and *DKK1* in ICAR samples and tended ($P \leq 0.10$) to have decreased mRNA abundance of *IRF1*, *IRF2*, *FOXL2*, and *PGR* in CAR samples, and *HOXA10* and *PAQR7* in ICAR samples. In contrast, MIX-supplemented heifers had increased ($P < 0.05$) mRNA abundance of *MSTN* in ICAR samples and an increase in conceptus length (ISe: 17.45 ± 3.08 cm vs. MIX: 25.96 ± 3.95 cm; $P = 0.05$). Notably, myostatin increases glucose secretion into histotroph and contributes to advanced conceptus development. This advancement in conceptus development occurred in the presence of similar concentrations of serum P4 ($P = 0.88$) and whole blood Se ($P = 0.07$) at MRP.

Lay Summary

In regions with soils deficient in selenium (**Se**), it is recommended that this trace mineral is supplemented to the diet of forage-grazing cattle. We have previously reported that the form of Se supplemented to cattle affects the function of multiple tissues, including the testis, liver, ovary, and pituitary. The objective of this study was to determine how the form of Se supplemented to heifers to achieve a Se-adequate status affects endometrial function and development of the conceptus at maternal recognition of pregnancy (**MRP**). Heifers were supplemented with the industry standard, an inorganic form of Se (**ISe**), or a 1:1 mix of organic and inorganic forms (**MIX**), with the reproductive tract recovered on day 17 of pregnancy. Real-time PCR was performed to determine the relative abundance of targeted mRNA transcripts in caruncular (**CAR**) and intercaruncular (**ICAR**) endometrial samples. The form of supplemental Se affected the abundance of multiple progesterone-induced and interferon-stimulated mRNA transcripts in CAR and ICAR samples, as well as the length of the conceptus that was recovered at MRP (day 17). Overall, our results indicate differences in endometrial function and increased development of the conceptus in cattle provided with MIX vs. ISe, suggesting that the MIX form of supplemental Se may increase fertility in cattle grazing soils deficient in this trace mineral.

Key words: conceptus, endometrium, maternal recognition of pregnancy, progesterone, selenium

Abbreviations: CAR, caruncular;CL, corpus luteum;ICAR, intercaruncular;IFNT, interferon tau;ISe, inorganic selenium;ISG, interferon-stimulated genes;LP, luteal phase;MIX, mixed selenium;mRNA, messenger RNA;MRP, maternal recognition of pregnancy;OSe, organic selenium;P4, progesterone;PBS, physiologically buffered saline;$PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$;PR, progesterone receptors;qPCR, real-time polymerase chain reaction;RIA, radioimmunoassay;Se, selenium;TRT, treatment

Introduction

The majority of producers in the southeast United States have forages and grains that are low (<0.05 ppm) to variable (~50% contain >0.1 ppm) in selenium (Se) (Ammerman and Miller, 1975). Therefore, it is not unexpected that producers in the southeast have the highest proportion of cattle classified as Se-deficient compared with other geographical regions (Dargatz and Ross, 1996). In cattle, deficiencies in Se have been demonstrated to decrease immunity (Erskine et al., 1989), growth (Gleed et al., 1983), and fertility (McClure

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et al., 1986). In the feed industry, Se is commonly supplemented in the inorganic (**ISe**) dietary form of sodium selenite or sodium selenate (Podoll et al., 1992). However, research has demonstrated lower concentrations of Se in whole blood in cattle supplemented with ISe compared with cattle supplemented with organic (**OSe**) Se (Sel-Plex; Alltech, Inc., Nicholasville, KY, USA; Patterson et al., 2013; Cerny et al., 2016a; Jia et al., 2018).

We previously reported increased early luteal phase (**LP**) concentrations of progesterone (**P4**) on day 6 (Cerny et al., 2016a) and on day 7 (Carr et al., 2020) of the estrous cycle in cows supplemented with a 1:1 combination (**MIX**) of ISe (sodium selenite; Prince Agri Products, Inc. Quincy, IL, USA) and OSe (SEL-PLEX; Alltech, Inc.) compared with cows supplemented with ISe alone. Moreover, we have recently reported that this increase in P4 could be explained, in part, by increased cholesterol biosynthesis (Crites et al., 2022) and increased cholesterol uptake (Carr et al., 2022) in corpora lutea recovered from MIX- vs. ISe-supplemented cows. Progesterone plays a crucial role in creating a uterine environment that favors advanced conceptus elongation (Lonergan, 2011). Supplementing dairy cows during the pre- and postpartum periods with a Se-enriched yeast has also been reported to increase postpartum plasma concentrations of P4 on days 32 to 34 when compared with providing no supplemental Se (Kamada, 2017), with concentrations of progesterone in postpartum cattle closely associated with rates of conception and early embryonic development (Breuel et al., 1993; Inskeep, 2004). Higher concentrations of progesterone following parturition can reduce the incidence of a short-lived corpus luteum (**CL**) affecting the ability of each cow to maintain pregnancy (Breuel et al., 1993; Inskeep, 2004). Additionally, elevated concentrations of progesterone at 17 to 25 d postpartum in cattle receiving norgestomet prior to synchronous weaning had significantly more embryos present at day 35 after breeding compared with control animals without progestin treatment (Breuel et al., 1993).

Progesterone acts on the uterus to indirectly stimulate preimplantation blastocyst growth and elongation (Garrett et al., 1988; Mann and Lamming, 2001). The elongating conceptus must secrete a chemical signal to prevent the uterine release of luteolytic pulses of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and therefore luteal regression, a process referred to as "maternal recognition of pregnancy" (**MRP**; Short, 1969). In cattle, that signal is interferon tau (**IFNT**; Lewis et al., 1979) and MRP occurs around days 16 to 17 after breeding (Spencer, 2013). In the uterus, P4 acts on the endometrium as a differentiation factor (Cummings and Yochim, 1984) and stimulates secretions of the glandular epithelium. Increased concentrations of P4 immediately following conception have been associated with advanced conceptus elongation (Garrett et al., 1988; Carter et al., 2008) and increased IFNT production (Mann and Lamming, 2001). Additionally, IFNT stimulates a number of genes in a cell-specific manner within the endometrium that are implicated in uterine receptivity and conceptus development (Spencer et al., 2007). Together, these proteins secreted by the uterus provide an environment that supports early embryonic development (Niswender et al., 2000).

The early embryo is nourished by histotroph from the uterine glands that are located in the intercaruncular endometrium (**ICAR**; Atkinson et al., 1984), with histotroph shown to be crucial for the development of the conceptus (Gray et al., 2001). Additionally, small aglandular caruncular (**CAR**) areas of stromal origin are scattered over the endometrium surface (Mansouri-Attia et al., 2009). The CAR areas are present in the cyclic endometrium and fuse with the fetal cotyledons to form placentomes in pregnant animals (Atkinson et al., 1984). During the pivotal time period encompassing MRP, failure or delays in trophoblast elongation and/or embryonic development result in loss of pregnancy possibly due to suboptimal histotroph (Wiltbank et al., 2016). Although rates of fertilization in cattle are high (Diskin and Morris, 2008), pregnancy losses average approximately 30% from days 8 to 27 of gestation, a period of time encompassing embryo elongation and MRP (Wiltbank et al., 2016). The loss of pregnancy at any stage of gestation has a clear negative impact on the reproductive performance and profit potential in beef and dairy operations.

The objectives of this study were to investigate the effects of a form of supplemental Se on gene expression in the bovine endometrium and on the growth of the developing conceptus on day 17 of pregnancy, further contributing to our long-term goal of elucidating form of Se-regulated processes affecting fertility. We hypothesized that the form of Se supplemented to heifers would alter gene expression in both the CAR and ICAR bovine endometrium, with MIX creating an environment that favors conceptus development. More specifically, we hypothesized that conceptus development would be more advanced in heifers supplemented with MIX vs. ISe. Providing supplemental minerals is a production practice that is easily implemented by producers and incorporating Se as MIX would be a simple transition with the potential to increase fertility in their herds.

Material and Methods

The experimental procedures in this project were approved by the Institutional Animal Care and Use Committee at the University of Kentucky, protocol number 2017-2828.

Animals and experimental procedure

Fall-born, Angus-cross yearling heifers (N = 20) were randomly selected from preexisting, Se-form-specific cow herds as previously described (Patterson et al., 2013; Matthews et al., 2014; Cerny et al., 2016a, 2016b) and were housed at the C. Oran Little Research Center at the University of Kentucky. At the beginning of this experiment, heifers (9.5 to 11.5 mo of age, August 2020) received ad libitum access to a basal mineral mix with no exogenous source of Se for a 45-d period of Se-depletion. This was followed by a 45-d period of repletion where all heifers had ad libitum access to a mineral mix with 35-ppm Se as ISe to return systemic blood Se in all heifers to adequate concentrations (National Academies of Sciences, Engineering, and Medicine, 2016). Following the period of Se repletion, heifers were randomly assigned to have at least 90 d of ad libitum access to a basal mineral mix containing 35-ppm Se as ISe (n = 10; ISe; sodium selenite; Prince Agri Products, Inc.) or as 1:1 combination of ISe and OSe (n = 10; MIX; SEL-PLEX; Alltech, Inc.) as described by (Carr et al., 2022). Throughout the depletion and repletion periods, all heifers grazed a common, novel, nontoxic endophyte-infected tall fescue (Lacefield MaxQ II) pasture and during the Se-specific treatment periods, heifers grazed toxic endophyte-infected tall fescue pastures with the addition of a common corn silage diet.

To verify that heifers maintained an adequate status of total blood Se during this trial, whole blood was collected from each heifer at the start and endpoint of the depletion and repletion periods and bimonthly until the end of the experiment for the determination of total whole blood concentrations of Se (Patterson et al., 2013; Cerny et al., 2016b).

Experimental regimen and tissue collection

Heifers were supplemented with their respective mineral treatment for at least 90 d (range 103 to 173 d) before being randomly injected with one or two doses of dinoprost tromethamine (25 mg, Lutalyse, Zoetis, Parsippany, NJ, USA) to induce regression of the CL and then monitored for behavioral estrus (day 0), twice daily, using visual appraisal and electronic cow monitoring technology (CowManager, Gerverscop 9, The Netherlands). At detected estrus (0 h), the presence of a preovulatory follicle was confirmed via transrectal ultrasonography using a 5 to 8 MHz linear transducer (Ibex Pro, E.I. Medical Imaging, Loveland, CO, USA) prior to insemination. Heifers were inseminated at 0, 12, and 24 h after detected estrus using frozen semen from a single bull with known high fertility. On days 0, 5, 6, 7, 8, 11, 14, and 17 approximately 8 mL of blood was collected into additive-free tubes (Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ) by jugular venipuncture for retrieval and quantification of serum P4 via radioimmunoassay. Additionally, on days 0 and 17, 8 mL of whole blood was collected by jugular venipuncture for the subsequent determination of concentrations of whole blood Se at insemination and sample collection. The presence and diameter of the CL were determined by transrectal ultrasonography on the morning of day 17 prior to the transport of heifers to the University of Kentucky Meat Laboratory.

On day 17 after insemination, the ovaries and uterus were collected from each heifer after euthanasia by captive bolt stunning and exsanguination at the USDA inspected University of Kentucky Meat Laboratory. Reproductive tracts were immediately removed, placed on ice, and processed for the collection of the conceptus and tissue samples. Specifically, the uterine horn contralateral to the CL was ligated approximately 4 cm from the uterine bifurcation and the ipsilateral ovary bearing the CL was removed. Subsequently, an artificial insemination sheath (Alpha sheath, IMV Technologies USA, Maple Grove, MN, USA) was passed through the cervix and 20 mL of ice-cold physiologically buffered saline (**PBS**) was infused into the uterine horn, massaged gently, and exited through an incision at the tip of the ipsilateral uterine horn. Uterine luminal flushing media and the conceptus were recovered in a sterile 100 by 15-mm Petri dish. If no conceptus was recovered in the first flush, then an additional flush of 20 mL of PBS was performed. If no conceptus was recovered after flushing four consecutive times, heifers were determined to be nonpregnant and were removed from the experiment. A complete, intact conceptus was recovered from MIX (n = 6) and ISe (n = 6) heifers, and only heifers with an intact conceptus that was recovered were included in further analyses. Digital images of the conceptuses were captured over 6.35 mm grid paper and conceptus lengths were measured using the Digimizer (version 5.6.0) software program, by two independent researchers. Conceptus lengths were averaged to determine a composite conceptus length to be used in statistical analyses.

Endometrial samples were randomly collected from the uterine horn ipsilateral to the ovary bearing the CL for gene expression analysis. The ipsilateral uterine horn was longitudinally opened by scissors and CAR and ICAR endometrium samples were collected by the same individual for all animals using an 8 mm biopsy punch (Integra LifeSciences Production Corporation, Mansfield, MA, USA). After collection, the conceptus, CAR, and ICAR samples were snap-frozen in liquid nitrogen and stored at −80 °C to be used for RNA extraction and the determination of transcript expression by real-time PCR (**qPCR**).

RNA extraction

Total RNA was extracted from ~200 mg of frozen CAR and ICAR endometrial tissue using TRIzol Reagent (Invitrogen Corporation, Carlsbad, CA) following the manufacturer's instructions. The purity and concentration of total RNA samples were analyzed using a NanoDrop ND-100 Spectophotometer (NanoDrop Technologies, Wilmington, DE, USA). All samples had high purity, with 260/280 absorbance ratios of 1.88 or greater.

qPCR analysis

The relative quantification of mRNA for genes of interest was performed using qPCR using standard procedures in our laboratory, as described previously (Cerny et al., 2016b; Carr et al., 2022). Briefly, 1 µg of each RNA sample was reverse transcribed to cDNA using the SuperScript IV VILO Master Mix with ezDNAse Enzyme (Invitrogen by Thermo Fisher Scientific, Vilnius, Lithuania). Additionally, a no reverse-transcription control sample was transcribed and analyzed via qPCR to ensure that products from the targeted transcripts were not obtained from genomic DNA.

The relative abundance of mRNA encoding interferon-stimulated transcripts: *ACKR3*, *IFIT3*, *IRF1*, *IRF2*, *ISG15*, *MSX1*, *MX1*, *MX2*, *OAS1*, *OAS2*, and *RSAD2* and the P4-induced transcripts: *DGAT2*, *DKK1*, *FABP3*, *FGF2*, *MSTN*, *SLC1A5*, *SLC1A3*, *SLC46A3*, *FOXL2*, *IHH*, *HOXA10*, and *IGFBP1* was quantified. Next, the relative abundance of P4-associated enzymatic transcripts: *PTGS2* and *PGES* and the receptor transcripts: *OXTR*, *ESR1*, *PGR*, *PGRMC1*, *PGRMC2*, *PAQR5*, *PAQR7*, *PAQR8*, *EP1*, *EP2*, *EP3*, *EP4*, *PGFR*, *IFNAR1*, and *IFNAR2* was quantified. Primer sequences used and GenBank accession numbers are listed in Tables 1 and 2. The qPCR procedures were performed using the Bio-Rad CFX Maestro thermal cycler (Bio-Rad, Hercules, CA, USA) with iTaq Universal SYBR Green Supermix (Bio-RAD). A total volume of 25 µL was used in each qPCR reaction containing 5 µL of cDNA, 1 µL of a 10 µM stock of each primer (forward and reverse), 12.5 µL of 2 × SYBR Green PCR Master Mix, and 5.5 µL of nuclease-free water. The relative amount of each transcript was calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Primer sets for genes of interest were designed and obtained from the NCBI Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) against RefSeq.

All cDNA products were validated via DNA sequencing for verification of target at ACGT, Inc. (Wheeling, IL, USA). The resulting sequences were then compared with the NCBI RefSeq mRNA sequences used for primer templates. The primer pair design, amplicon length of the product, and product identity for each targeted transcript are shown in Tables 1 and 2. Three constitutively expressed genes (*ACTB*, *GAPDH*, and *SDHA*) with CT values not affected ($P > 0.05$) by Se-form

Table 1. Primer sets and product identities of real-time PCR analysis of progesterone-induced and interferon-stimulated genes

Gene	Gene name	Accession number[1]	Primer design (5′ to 3′) direction	Amplicon length, bp	Product identity[2], %
Progesterone-induced transcripts					
DGAT2	*Bos taurus diacylglycerol O-acyltransferase 2*	NM_205793.2	F: AACACACCCAAGAAAGGTGGC R: GCTTACTTCTGTGGCCTCTGT	204	100
DKK1	*Bos taurus dickkopf WNT signaling pathway inhibitor 1*	NM_001205544.1	F: GGCAGCAAGTACCAGACCAT R: AGAAGGCATGCATATCCCGTT	207	100
FABP3	*Bos taurus fatty acid binding protein 3*	NM_174313.2	F: TGAAGTCACTCGGTGTCGGT R: TCAACCATCTCCCGCACAAG	271	100
FGF2	*Bos taurus fibroblast growth factor 2*	NM_174056.4	F: AAGCGGCTGTACTGCAAGAA R: ACACTCGTCTGTAACACATTTAGAA	216	100
FOXL2	*Bos taurus forkhead box L2*	NM_001031750.1	F: GCAGAAGCCCCCATACTCTT R: GGTCCAGCGTCCAGTAGTTG	239	100
HOXA10	*Bos taurus homeobox A10*	NM_001105017.1	F: TTTCGGAAATGTGTCAAGGCAA R: CGGATCCGGTTTTCTCGGTT	262	100
IGFBP1	*Bos taurus insulin like growth factor binding protein 1*	NM_174554.3	F: CAGCGATGAGGCTACAGATAC R: GCTGCTCCCTGGCTAATCTG	257	99
IHH	*Bos taurus Indian hedgehog signaling molecule*	NM_001076870.2	F: GCCAACAATCACACTGAGCC R: CCAAGCTGTGAAACAGTCGC	274	100
MSTN	*Bos taurus myostatin*	NM_001001525.3	F: TGCCCACGGAGTCTGATCTT R: TGCCTGGGTTCATGTCAAGT	237	100
SLC1A3	*Bos taurus solute carrier family 1 member 3*	NM_174600.2	F: GGGCGCCGTGATAAACAATG R: GAGGGGCGTACCACATGAT	242	100
SLC1A5	*Bos taurus solute carrier family 1 member 5*	NM_174601.2	F: CAAGGAGGTGCTCGATTCGT R: ACAGGGGCGTACCACATGAT	306	100
SLC46A3	*Bos taurus solute carrier family 46 member 3*	NM_001103303.2	F: TCTACTGAGCAAGGGACCAT R: CCCGTATTCCTGCTGACGTA	200	100
Interferon-stimulated transcripts					
ACKR3	*Bos taurus atypical chemokine receptor 3*	NM_001098381.2	F: TACTCAGAGCCGGGGAACTT R: TGTAGCAGTGCGTGTCGTAG	226	99
IFIT3	*Bos taurus interferon induced protein with tetratricopeptide repeats 3*	NM_001075414.1	F: ATTCTGAAGCAGGCCGTTGA R: TCCAGTGCCCTTAGCAACAG	224	100
ISG15	*Bos taurus ISG15 ubiquitin like modifier*	NM_174366.1	F: CCATCCTGGTGAGGAACGAC R: GAACACGGTGCACCCCTTCA	200	99
MSX1	*Bos taurus msh homeobox 1*	NM_174798.2	F: CCATTTCTCGGTGGGAGGAC R: GTACTGCTTCTGGCGGAACT	241	100
MX1	*Bos taurus MX dynamin like GTPase 1*	NM_173940.2	F: ACATGATCGTCAAGTGCCGT R: ACAGGGGCAGAGTTTTACAAATG	201	100
MX2	*Bos taurus MX dynamin like GTPase 2*	NM_173941.2	F: GCTCCAGAAGGCCATGGAAAT R: AACCACGCCGTAAATCTGGT	208	100
OAS1Z	*Bos taurus 2′,5′-oligoadenylate synthetase 1*	NM_001029846.2	F: GGAGACGTGCTTCCAAGAGT R: TCTTCAGTCACCTGAGCTTGTG	381	99
OAS2	*Bos taurus 2′-5′-oligoadenylate synthetase 2*	NM_001024557.1	F: ACTGGTTTCAAAAGTGCCAGG R: CAGCCAGCAGGTGTTATCCA	314	98
RSAD2	*Bos taurus radical S-adenosyl methionine domain containing 2*	NM_001045941.1	F: GTGGTTCCAGAAGTACGGTGA R: AACCGTTCCGCTTCTCTCAG	315	100

[1]These contents are associated with each gene symbol and are the accession numbers of the sequences retrieved from the NCBI RefSeq database for designing primers and probes.
[2]All qPCR products were validated by sequencing. The identity values (%) presented are the base pair ratios between the total amplicon length and the number of identical base pairs.

treatment were used to normalize the relative mRNA expression to the geometric mean of the three. For qPCR analysis, n = 6 and 6 for ISe and MIX treatments, respectively. Data were normalized to levels of ISe expression and all reactions were performed in duplicate.

Se and P4 analysis

Total blood Se was determined by the University of Kentucky's Veterinary Diagnostics Laboratory (Lexington, KY) and Se was quantified using an Agilent 7900 inductively coupled plasma-mass spectrometer, as described previously (Wahlen et al., 2005).Concentrations of P4 were quantified in samples of serum by a commercially available competitive RIA without extraction (ImmuChem Coated Tube Progesterone 125-I RIA Kit, MP Biomedicals, Costa Mesa, CA, USA) according to the manufacturer's instructions. There was one assay performed for analysis of serum P4 with an intra-assay CV of 10.16%.

Table 2. Primer sets and product identities for real-time PCR analysis of progesterone-associated enzymatic and receptor transcripts, as well as reference genes

Gene	Gene name	Accession number[1]	Primer design (5′ to 3 ′) direction	Amplicon length, bp	Product identity [2], %
Enzymatic transcripts					
PTGES	*Prostaglandin E synthase*	NM_174443.2	F: CGCTGCTGGTCATCAAAATGT R: GGTCTCCATGTCATTCCGGT	173	97
PTGS2	*Prostaglandin-endoperoxide synthase 2*	NM_174445.2	F: CCCATGGGTGTGAAAGGGAG R: TCCACCCCATGGTTCTTTCC	203	100
Receptor transcripts					
IRF1	*Bos taurus interferon regulatory factor 1*	NM_001191261.2	F: ACAGCCCCGATACCTTCTCT R: CTTCCCATCCACGCTTGTCT	338	100
IRF2	*Bos taurus interferon regulatory factor 2*	NM_001205793.2	F: TGGGCCATCCATACAGGAAA R: CCGTCCAGATGTGACTGTCC	383	99
OXTR	*Bos taurus oxytocin receptor*	NM_174134.2	F: GCAGCTTCTGTGGGACATCA R: TCCACGTGATGTAGGCCTTG	326	99
ESR1	*Estrogen receptor 1*	NM_001001443.1	F: ATGGCCATGGAATCTGCCAA R: GGTCTTTCCGTATTCCGCCT	256	99
PGR	*Nuclear progesterone receptor*	NM_001205356.1	F: CCCACAGGAGTTTGTGAAGC R: AGTGCCCGGGACTGGATAAA	291	99
PGRMC1	*Progesterone receptor membrane component 1*	NM_001075133.1	F: GGCCGTATGGAGTCTTTGCT R: TTGTCTGAGTACACGGTGGG	217	100
PGRMC2	*Progesterone receptor membrane component 2*	NM_001099060.1	F: GCTTGCGGTCAATGGGAAAG R: GACGGTTCTTCCCCTGGTTT	264	99
EP1	*Prostaglandin E receptor 1*	NM_001192148.1	F: GGCCGCTGTTTTTGGCCGTG R: CCTCCATGGCTGCCCTTGGC	142	100
EP2	*Prostaglandin E receptor 2*	NM_174588.2	F: GCTTCATCGGACACAAGCAG R: CTCCGCCATGGATACCCTTT	197	100
EP3	*Prostaglandin E receptor 3*	NM_181032.1	F: CGCCGTTGCTGATAATGATGT R: GTCCTTTCAAAAGCTGGCAA	204	100
EP4	*Prostaglandin E receptor 4*	NM_174589.2	F: CGGGACCAATGCATCATCCT R: TTGGCCCTTCAAGTAGGTGG	241	100
PAQR5	*Progestin and adipoQ receptor family member 5 (mPR_{γ})*	XM_024997926.1	F: GGTTCTTCTCGTGGAGGTTTGT R: GTTCCTGGACATGGAGCTGAA	151	96
PAQR7	*Progestin and adipoQ receptor family member 7 (mPR_{α})*	NM_001038553.1	F: CCGGCGGTCCATCTATGA R: CCACCCCCTTCACTGAGTCTT	159	99
PAQR8	*Progestin and adipoQ receptor family member 8 (mPR_{β})*	NM_001101135.2	F: TGTAGCCTTGCGAGACACAG R: CAGCATCGCAGAAGAATGCC	214	100
PTGFR	*Prostaglandin F receptor*	NM_181025.3	F: TGGTGTTCTCTGGTCTGTGC R: GGCTAGGAGCCCCAGAAAAG	293	100
IFNAR1	*Bos taurus interferon alpha and beta receptor subunit 1*	NM_174552.2	F: ACAGGCGGAATAAAGGGAGC R: GGCTGATCGGAGAAATACTCGT	220	99
IFNAR2	*Bos taurus interferon alpha and beta receptor subunit 2*	NM_174553.2	F: CCCAGACGAGAATCAGAGTCAT R: TGGGGAGCTGCCTCATTTTC	299	100
Housekeeping Transcripts					
ACTB	*Actin beta*	NM_173979.3	F: GAGCGGGAAATCGTCCGTGAC R: GTGTTGGCGTAGAGGTCCTTGC	278	99
GAPDH	*Glyceraldehyde 3-phosphate dehydrogenase*	NM_001034034.2	F: ACATCAAGTGGGGTGATGCT R: GGCATTGCTGACAATCTTGA	201	97
SDHA	*Succinate dehydrogenase complex flavoprotein subunit A*	NM_174178.2	F: GCAGAACCTGATGCTTTGTG R: CGTAGGAGAGCGTGTGCTT	185	99

[1]These contents are associated with each gene symbol and are the accession numbers of the sequences retrieved from the NCBI RefSeq database for designing primers and probes.
[2]All qPCR products were validated by sequencing. The identity values (%) presented are the base pair ratios between the total amplicon length and the number of identical base pairs.

Statistical analysis

Data are presented as least square means (±SEM) with individual heifer as the experimental unit. Data were analyzed for normal distribution and homogeneity. When appropriate, data were transformed for normality by natural log transformation.

To determine the effect of the form of Se on concentrations of systemic Se and P4, data were analyzed using the PROC

MIXED procedure of SAS statistical software package (version 9.4; SAS Institute, Inc. Cary, NC, USA) as an ANOVA with repeated measures. The form of dietary Se was considered the fixed effect for both. The effect of the form of Se supplementation on conceptus length was analyzed as a one-tailed student's *t*-test, using the PROC TTEST procedure of SAS statistical software package (version 9.4; SAS Institute, Inc.).

The effect of the form of Se supplementation on the relative abundance of CAR and ICAR mRNA transcripts was analyzed as a one-way ANOVA using the PROC GLM procedure of SAS statistical software package (version 9.4; SAS Institute, Inc.). For all analyses, $n = 6$ for ISe and MIX treatments and significance was declared at $P < 0.05$, and a tendency to differ was declared when $P \leq 0.10$.

Results

Concentrations of whole blood Se

Heifers were maintained on form of Se-specific treatments (ISe vs. MIX) that provided adequate concentrations of whole blood Se (Gerloff, 1992; Dargatz and Ross, 1996) for the duration of the treatment and sample collection periods of this trial (Figure 1). There was an effect of time ($P < 0.01$) and tended to be the main effect of the form of Se ($P = 0.07$), but there was no significant treatment by time interaction ($P = 0.51$).

Progesterone

Serum collected from heifers on days 0, 5, 6, 7, 8, 11, 14, and 17 of gestation was used to determine concentrations of P4. Previous studies from our lab indicate an increase of ~1 ng/mL on days 6 and 7 of the estrous cycle (Cerny et al., 2016a; Carr et al., 2020). In the current study, concentrations of P4 were not affected ($P = 0.88$) by the form of Se-treatment (Table 3). There was an effect of the day ($P < 0.01$), but there was no treatment by day interaction ($P = 0.77$).

Conceptus length

The form of Se affected conceptus length on day 17 of gestation. Conceptus length was increased ($P = 0.05$) in heifers supplemented with MIX (25.96 ± 3.95 cm) compared with ISe (17.45 ± 3.08 cm; Figure 2). A representative image of a collected conceptus is shown in Figure 3.

qPCR analysis of selected mRNA transcripts

The relative abundance of 40 mRNAs encoding interferon-stimulated transcripts, P4-induced transcripts, P4-associated enzymatic transcripts, and receptor transcripts was analyzed in both CAR and ICAR samples via qPCR. Of the 11 interferon-stimulated transcripts (Table 4), mRNA encoding *IFIT3*, *ISG15*, *MX1*, *OAS2*, and *RSAD2* was decreased ($P < 0.05$) in CAR retrieved from MIX- vs. ISe-supplemented heifers (Figure 4). Similarly, the relative abundance of *IRF1* and *IRF2* tended ($P \leq 0.10$) to be lower in CAR retrieved from MIX- vs. ISe-supplemented heifers (Figure 4).

Of the 12 targeted P4-induced transcripts (Table 5), mRNA encoding *DGAT2* and *FGF2* was decreased ($P < 0.05$) in CAR retrieved from MIX- vs. ISe-supplemented heifers (Figure 5). Additionally, the relative abundance of *FOXL2* tended ($P < 0.10$) to be lower in CAR retrieved from MIX- vs. ISe-supplemented heifers. Similarly, MIX-supplemented heifers had decreased ($P < 0.05$) abundance of mRNA encoding *DKK1* and tended ($P \leq 0.10$) to have an increased abundance of mRNA encoding *HOXA10* in ICAR samples, compared with heifers supplemented with ISe. In contrast, MIX-supplemented heifers had an increased ($P < 0.05$) abundance of mRNA encoding *MSTN* in ICAR compared with ISe-supplemented heifers (Figure 5). Of the 17 targeted receptor transcripts (Table 6), mRNA encoding the nuclear P4 receptor (*PGR*) in CAR samples and the P4 membrane receptor (*PAQR7*) in ICAR samples tended ($P \leq 0.10$) to be decreased in MIX- vs. ISe-treated heifers (Figure 6).

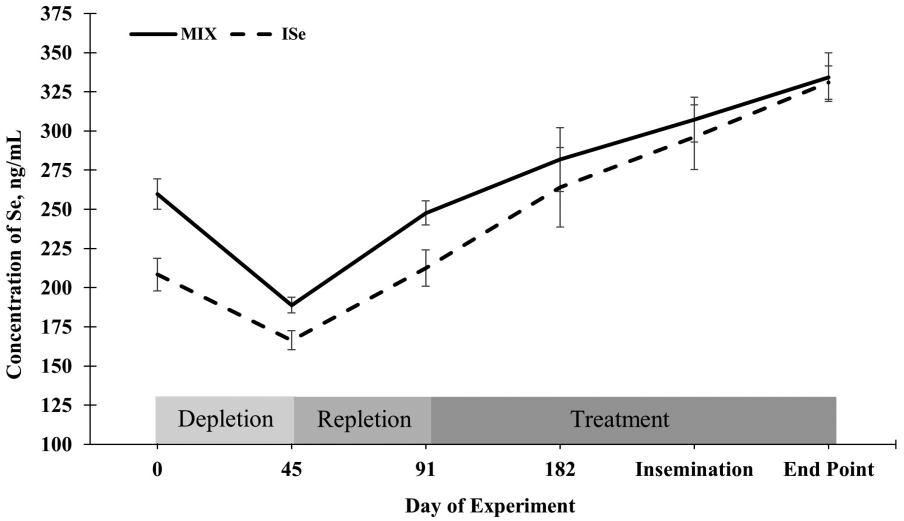

Figure 1. Concentration of total Se in whole blood. Effect of form of Se on whole blood concentrations of Se (ppm, LS mean ± SEM) in heifers supplemented with either ISe (sodium selenite, $n = 6$) or a 1:1 combination (MIX) of ISe and OSe (Sel-Plex, $n = 6$). Data were analyzed as an ANOVA with repeated measures. Whole blood Se tended to be affected by treatment ($P = 0.07$) and was affected by time ($P < 0.01$), but there was no significant treatment by time interaction ($P = 0.51$).

Discussion

The aim of this study was to investigate the effect of the form of supplemental Se on the uterine endometrium and on the development of the conceptus at the time of MRP in cattle. More specifically, we hypothesized that supplementation with MIX vs. the industry standard of ISe would affect endometrial gene expression and increase the length of the developing conceptus at MRP. We have previously reported that MIX increases early LP concentrations of P4 (Cerny et al., 2016a; Carr et al., 2020), and that this increase in P4 is likely the result of MIX induced increases in luteal transcripts associated with cholesterol biosynthesis (Crites et al., 2022) and cholesterol uptake (Carr et al., 2022). Increasing early LP P4 stimulates endometrial functions necessary for conceptus growth (Spencer and Bazer, 2002), leading to advanced conceptus elongation (Garrett et al., 1988; Carter et al., 2008) and increased production of IFNT (Mann and Lamming, 2001).

In the present study, the form of Se (treatment) did not affect circulating concentrations of P4. The inability to detect a MIX induced increase in P4, as reported in our earlier studies (Cerny et al., 2016a; Carr et al., 2020), is likely limited by the number of animals used in the present study; however, heifers in the present study were also managed on both nontoxic and toxic endophyte-infected tall fescue pastures and received a common corn silage diet during the winter months. Cows used in previous studies from our lab were grazing toxic endophyte-infected tall fescue pastures throughout (Cerny et al., 2016a; Carr et al., 2020). Previous research has demonstrated that heifers fed endophyte-infected fescue had lower LP concentrations of P4 than heifers consuming a diet containing endophyte-free fescue (Jones et al., 2003). Perhaps there is also a relationship between endophyte toxicity and the form of supplementary Se that accounts for the differences between these studies.

Successful pregnancy in mammals requires both a viable embryo and a receptive endometrium (Walker et al., 2010). Synchronous signaling between the endometrium and embryo during the preimplantation period is critical for normal embryo development, implantation of the embryo, and placentation (Wolf et al., 2003). Uterine receptivity has been demonstrated to be dependent on P4 (Mansouri-Attia et al., 2009). Additionally, uterine factors including enzymes, cytokines, growth factors, ions, hormones, glucose, transport proteins, and adhesion molecules, collectively termed "histotroph," have been shown to be mainly synthesized by the endometrial glands (Martal et al., 1997). Research has indicated that P4-induced changes in endometrial gene expression lead to changes in the composition of histotroph that are required for post-hatching conceptus survival and growth (Spencer et al., 2008).

The actions of P4 are mediated by P4 receptors (**PR**; Spencer and Bazer, 2002). PR are expressed in endometrial epithelia and stroma during the early to mid-LP, allowing direct regulation of genes by P4 (Spencer et al., 2008). However, continuous exposure of the endometrium to P4 negatively regulates

Table 3. Effect of form of Se on serum concentrations (ng/mL; LS mean ± SEM) of progesterone in heifers supplemented with either ISe (sodium selenite; *n* = 6) or a 1:1 combination (MIX) of ISe and OSe (Sel-Plex; *n* = 6)

Variable	Treatment		*P*-value [1]
	ISe LS mean ± SEM	MIX LS mean ± SEM	
Progesterone, ng/mL			
No. of heifers, *n*	6	6	
Day 0	0.11 ± 0.07	0.09 ± 0.07	0.40
Day 5	1.07 ± 0.18	1.12 ± 0.41	0.58
Day 6	1.52 ± 0.34	1.94 ± 0.27	0.49
Day 7	2.09 ± 0.23	2.28 ± 0.29	0.81
Day 8	2.54 ± 0.34	2.48 ± 0.56	0.83
Day 11	2.70 ± 0.47	2.99 ± 0.51	0.83
Day 14	3.96 ± 0.76	3.93 ± 0.77	0.83
Day 17	3.85 ± 0.86	3.54 ± 1.04	0.62

[1]*P*-values associated with ANOVA with repeated measures. Serum progesterone was not affected by treatment ($P = 0.88$) but was affected by day ($P < 0.01$), however there was no treatment by day interaction ($P = 0.77$).

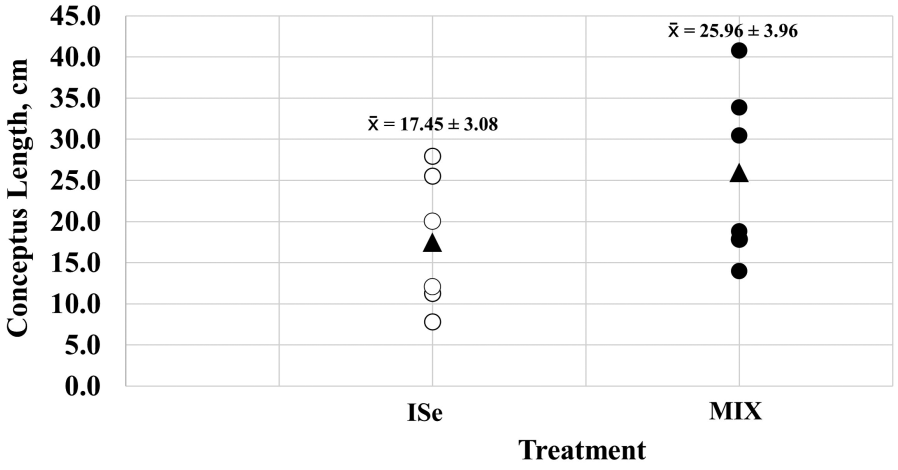

Figure 2. Effect of the form of Se on length of the conceptus (cm; LS mean ± SEM). Heifers were supplemented with either ISe (sodium selenite, *n* = 6) or a 1:1 combination (MIX) of ISe and OSe (Sel-Plex, *n* = 6). Recovered conceptuses are represented by individual dots in their respective treatments and within treatment, the mean is represented by a black triangle. Data were analyzed as a one-tailed Student's *t*-test. Length of the conceptus was affected by treatment ($P = 0.05$).

PR expression in the luminal and glandular epithelium, and the down-regulation of PR is temporally associated with the induction of many P4-stimulated genes (Spencer et al., 2004, 2008). Furthermore, the loss of PR is associated with the induction of numerous genes associated with cell adhesion (Spencer et al., 2008). Results from qPCR performed in the study herein indicated a tendency for the relative abundance of mRNA encoding the nuclear P4 receptor (*PGR*) and progestin and adipoQ receptor family member 7 (*PAQR7*) to be decreased in ICAR tissue recovered from MIX compared with ISe-supplemented heifers. Since PR are downregulated from continuous exposure to P4, this MIX-induced reduction in *PGR* and *PAQR7* mRNAs is consistent with the increased concentration of P4 previously reported by our laboratory (Cerny et al., 2016a; Carr et al., 2020).

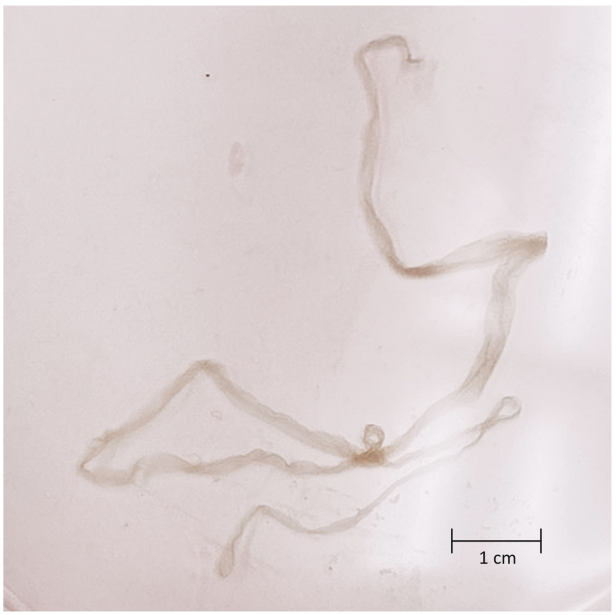

Figure 3. Representative image of a conceptus collected on day 17 of pregnancy.

As reviewed by Spencer et al. (2004), the paradigm of loss of PR in uterine epithelia immediately before implantation is common in sheep, cattle, pigs, and mice. Thus, regulation of endometrial epithelial function during the peri-implantation period must be directed in response to P4 by specific factors produced by PR-positive stromal cells (Cunha et al., 1985). For example, research has demonstrated that P4 regulates forkhead Box L2 (FOXL2) expression in the endometrium of ruminants and stimulates FOXL2 promoter activity through nuclear P4 receptors (Eozenou et al., 2020). Results from qPCR in the study herein revealed a MIX-induced decrease in mRNA abundance of *FOXL2*. In the bovine endometrium, a negative correlation between circulating concentrations of P4 and FOXL2 gene expression exists (Eozenou et al., 2012). FOXL2 appears to be important in the endometrium as well as a key gene involved in ovarian differentiation and maintenance of ovarian function (Eozenou et al., 2012; Georges et al., 2014; Elzaiat et al., 2017). Interestingly, it has been revealed that FOXL2 is a transcriptional repressor of the StAR protein gene (Pisarska et al., 2004). Importantly, StAR protein transports cholesterol to the inner mitochondrial membrane and is the rate-limiting step in steroidogenesis (Clark et al., 1994; Manna et al., 2016).

From the perspective of the maternal system, a key regulator of uterine function and the production of histotroph involves circulating concentrations of P4 (Wiltbank et al., 2016). Histotroph represents maternal contributions to uterine luminal fluid and is composed of glucose, fatty acids, and amino acids (Forde et al., 2014). Although gluconeogenesis does not occur in the uterus (Zimmer and Magnuson, 1990; Yánez et al., 2003), glucose is stored as glycogen (Demers et al., 1972; Greenstreet and Fotherby, 1973). It is likely

Table 4. Real-time PCR[1] identification of selected interferon-stimulated mRNAs from CAR and ICAR endometrium of heifers supplemented with 3 mg Se per d in vitamin–mineral mixes as sodium selenite (ISe, $n = 6$) or a 1:1 blend (MIX, $n = 6$) of ISe and OSe (SEL-PLEX)[2]

Gene	Gene name	CAR[3]				ICAR[3]			
		ISe	MIX	SEM	*P*-value	ISe	MIX	SEM	*P*-value
ACKR3[4]	*Bos taurus atypical chemokine receptor 3*	1.05	0.82	0.13	0.22	1.03	0.86	0.16	0.29
IFIT3[4]	*Bos taurus interferon induced protein with tetratricopeptide repeats 3*	1.08[a]	0.65[b]	0.13	0.04	1.07	0.85	0.15	0.37
IRF1[5]	*Bos taurus interferon regulatory factor 1*	1.05[x]	0.75[y]	0.11	0.08	1.03	0.94	0.12	0.58
IRF2	*Bos taurus interferon regulatory factor 2*	1.02[x]	0.83[y]	0.06	0.06	1.02	0.87	0.08	0.19
ISG15[4]	*Bos taurus ISG15 ubiquitin like modifier*	1.04[a]	0.76[b]	0.09	0.05	1.05	0.94	0.10	0.65
MSX1	*Bos taurus msh homeobox 1*	1.05	1.08	0.12	0.85	1.08	0.85	0.15	0.29
MX1[4]	*Bos taurus MX dynamin like GTPase 1*	1.00[a]	0.79[b]	0.06	0.03	1.02	0.94	0.08	0.53
MX2	*Bos taurus MX dynamin like GTPase 2*	1.03	0.79	0.10	0.11	1.04	0.82	0.12	0.24
OAS1Z	*Bos taurus 2′,5′-oligoadenylate synthetase 1*	1.02	0.82	0.15	0.38	1.04	0.94	0.23	0.75
OAS2[5]	*Bos taurus 2′-5′-oligoadenylate synthetase 2*	1.03[a]	0.67[b]	0.10	0.01	1.05	0.88	0.12	0.34
RSAD2[5]	*Bos taurus radical S-adenosyl methionine domain containing 2*	1.05[a]	0.57[b]	0.14	0.01	1.02	0.82	0.08	0.10

[1]Data are expressed as a ratio of MIX relative to ISe expression.
[2]Selenium was supplemented at 35 ppm as either inorganic (ISe; sodium selenite) or a 1:1 combination (MIX) of ISe and OSe (SEL-PLEX). Selenium was supplemented to treatment groups ad libitum.
[3]Values are LS means and SEM.
[4]ICAR natural log transformed due to lack of normality.
[5]CAR natural log transformed due to lack of normality.
[a,b]Means within a row that lack a common superscript differ ($P < 0.05$).
[x,y]Means within a row that lack a common superscript tend to differ ($P \leq 0.10$).

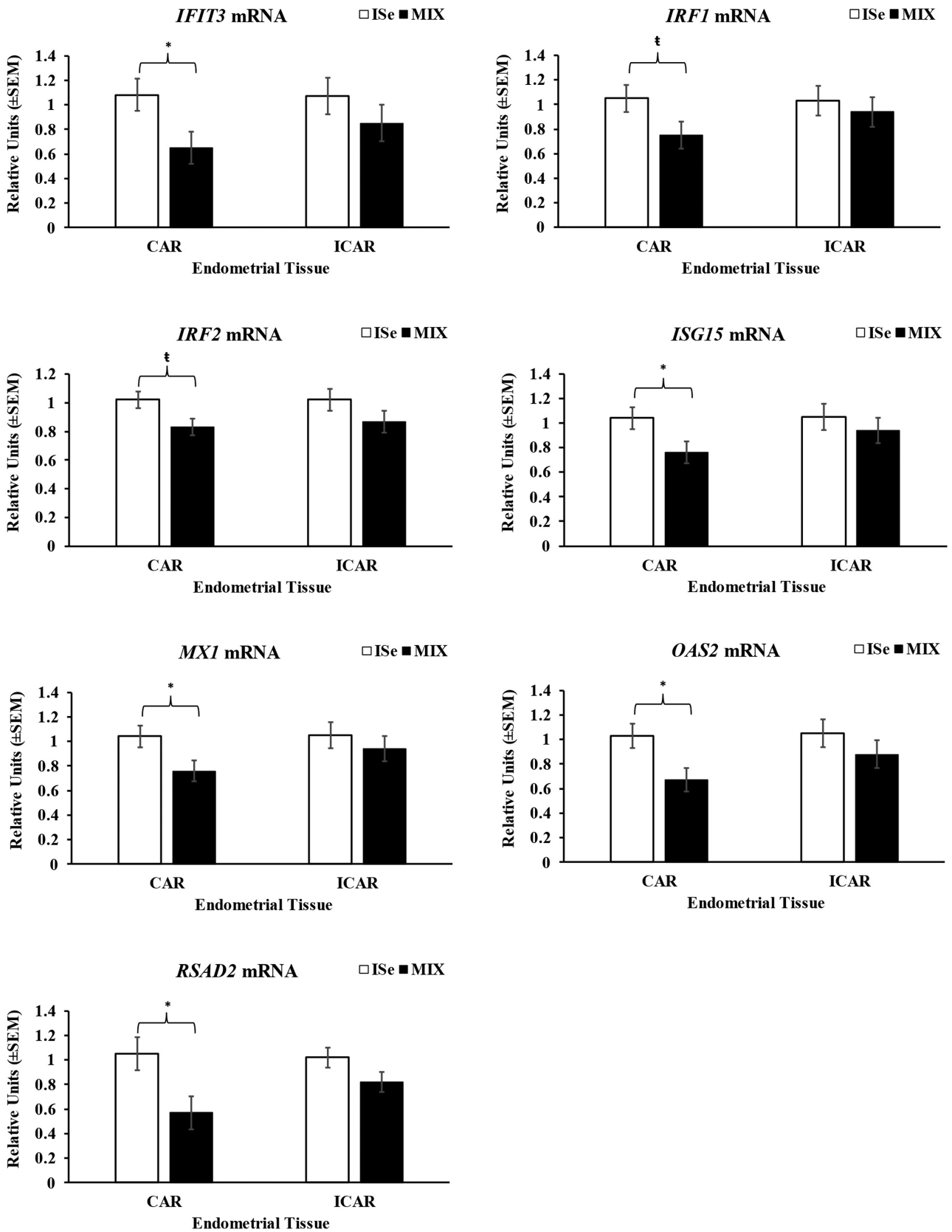

Figure 4. Effect of form of Se on the abundance of mRNA transcripts encoding interferon-stimulated genes in the endometrium of heifers supplemented with vitamin–mineral mixes containing Se as ISe (sodium selenite, $n = 6$) or a 1:1 combination (MIX) of ISe and OSe (Sel-Plex, $n = 6$). *P*-values are associated with ANOVA. Significant differences at $P < 0.05$ are indicated by an asterisk and tendencies at $P \leq 0.1$ are indicated by ŧ.

Table 5. Real-time PCR[1] identification of selected progesterone-induced mRNAs from CAR and ICAR endometrium of heifers supplemented with 3 mg Se per d in vitamin–mineral mixes as sodium selenite (ISe, *n* = 6) or a 1:1 blend (MIX, *n* = 6) of ISe and OSe (SEL-PLEX)[2]

Gene	Gene name	CAR[3]				ICAR[3]			
		ISe	MIX	SEM	*P*-value	ISe	MIX	SEM	*P*-value
DGAT2[4]	*Bos taurus diacylglycerol O-acyltransferase 2*	1.05[a]	0.64[b]	0.11	0.03	1.03	0.88	0.13	0.35
DKK1	*Bos taurus dickkopf WNT signaling pathway inhibitor 1*	1.07	0.93	0.17	0.55	1.10[a]	0.64[b]	0.15	0.05
FABP3[5]	*Bos taurus fatty acid binding protein 3*	1.38	1.38	0.43	0.92	1.77	1.82	0.46	0.94
FGF2	*Bos taurus fibroblast growth factor 2*	1.04[a]	0.71[b]	0.10	0.04	1.02	0.95	0.15	0.72
FOXL2	*Bos taurus forkhead box L2*	1.05[x]	0.70[y]	0.12	0.07	1.02	0.79	0.12	0.21
HOXA10	*Bos taurus homeobox A10*	1.04	0.91	0.10	0.33	1.07[x]	0.71[y]	0.14	0.10
IGFBP1[6]	*Bos taurus insulin like growth factor binding protein 1*	1.09	0.78	0.20	0.24	1.21	0.98	0.24	0.70
IHH	*Bos taurus Indian hedgehog signaling molecule*	1.16	1.23	0.24	0.83	1.08	0.89	0.16	0.43
MSTN	*Bos taurus myostatin*	1.05	1.45	0.18	0.14	1.03[a]	1.71[b]	0.14	0.01
SLC1A3[4]	*Bos taurus solute carrier family 1 member 3*	1.04	1.09	0.13	0.80	1.04	0.90	0.12	0.40
SLC1A5	*Bos taurus solute carrier family 1 member 5*	1.06	0.91	0.12	0.42	1.04	1.01	0.13	0.87
SLC46A3	*Bos taurus solute carrier family 46 member 3*	1.05	1.25	0.15	0.38	1.09	1.19	0.19	0.71

[1]Data are expressed as a ratio of MIX relative to ISe expression.
[2]Selenium was supplemented at 35 ppm as either inorganic (ISe; sodium selenite) or a 1:1 combination (MIX) of ISe and OSe (SEL-PLEX). Selenium was supplemented to treatment groups ad libitum.
[3]Values are LS means and SEM.
[4]ICAR natural log transformed due to lack of normality.
[5]CAR natural log transformed due to lack of normality.
[6]CAR and ICAR natural log transformed due to lack of normality.
[a,b]Means within a row that lack a common superscript differ ($P < 0.05$).
[x,y]Means within a row that lack a common superscript tend to differ ($P \leq 0.10$).

that uterine glycogen reserves are an important source of energy for pre-embryonic growth and development (Dean et al., 2014). Diacylglycerol O-acyltransferase homolog 2 (DGAT2) catalyzes the final step in the formation of triglyceride to acylcoenzyme A, and triglycerides are a potential energy source up to the blastocyst stage in cattle (Forde et al., 2009). In the present study, qPCR results revealed that MIX-supplemented heifers had decreased relative abundance of *DGAT2* in CAR tissue compared with heifers supplemented with ISe. Moreover, a P4-induced earlier increase in DGAT2 expression may increase the availability of triglyceride as an energy source for the developing conceptus (Forde et al., 2009). Additionally, P4 induction of myostatin (**MSTN**) may increase glucose secretion into histotroph, contributing to the advanced development of the conceptus after hatching (Forde et al., 2009). Importantly, MIX-supplemented heifers had an increased relative abundance of *MSTN* compared with ISe heifers in ICAR tissue. If DGAT2 had peaked prior to day 17 herein, and we were detecting a declining level of expression in MIX- vs. ISe-supplemented heifers, the results would suggest that conceptus recovered from MIX heifers may be more advanced in their development due to an earlier increase in P4, leading to an earlier increase in DGAT2, and thus increased MSTN on day 17 of gestation, however, this is speculative.

Progesterone stimulates and maintains endometrial functions necessary for conceptus growth, implantation, placentation, and development to term (Bazer, 1975; Bazer et al., 1979; Spencer and Bazer, 2002; Spencer et al., 2004). Heifers and ewes with lower concentrations of P4 in the early LP had smaller conceptuses that secreted less IFNT (Nephew et al., 1991; Mann and Lamming, 2001). Conversely, increased concentrations of P4 immediately following conception have been associated with advanced conceptus elongation (Carter et al., 2008) and increased IFNT production (Mann and Lamming, 2001). Although concentrations of P4 were similar between treatments at MRP in the present study, conceptus development was more advanced in MIX- vs. ISe-supplemented heifers. Importantly, the MIX-induced increase in conceptus development occurred in the presence of similar concentrations of whole blood Se. This MIX-induced increase in conceptus development is a salient finding of this research and could be, in part, due to the increased relative abundance of MSTN discussed above. It has been suggested that heifers with artificially increased concentrations of P4 soon after insemination had protein products required to advance conceptus development transported to the uterine lumen at an earlier stage than in normal P4 environments (Forde et al., 2009). Perhaps the increased concentrations of progesterone previously reported by our lab on days 6 (Cerny et al., 2016a) and 7 (Carr et al., 2020, 2022) and the numerical increase in concentrations of P4 on days 6 and 7 in MIX vs. ISe heifers in the present study allowed for an earlier induction of MSTN, ultimately contributing to the advanced conceptus development of MIX embryos recovered herein.

In addition to its antiluteolytic actions, IFNT produced by the conceptus acts on endometrial genes, known as interferon-stimulated genes (**ISGs**), in a specific spatial and temporal manner (Bazer et al., 2008, 2009). ISGs are implicated in uterine receptivity and conceptus development (Spencer et al., 2007). In a comparison of pregnant and cyclic heifers, differentially expressed genes identified on day 16 of pregnancy were due to the presence of the conceptus and the majority were expressed in response to IFNT produced by the conceptus (Forde et al., 2011). Unexpectedly, we observed a MIX-induced decrease in the expression of transcripts encoding numerous ISGs including *IFIT3*, *IRF1*, *IRF2*, *MX1*, *OAS2*, and *RSAD2* in CAR tissue.

Following the binding of IFNT to its receptors, IFNAR1 and IFNAR2, it initiates cell signaling via Janus activation kinases (JAKs) and tyrosine kinase 2 (TYK2; Bazer et

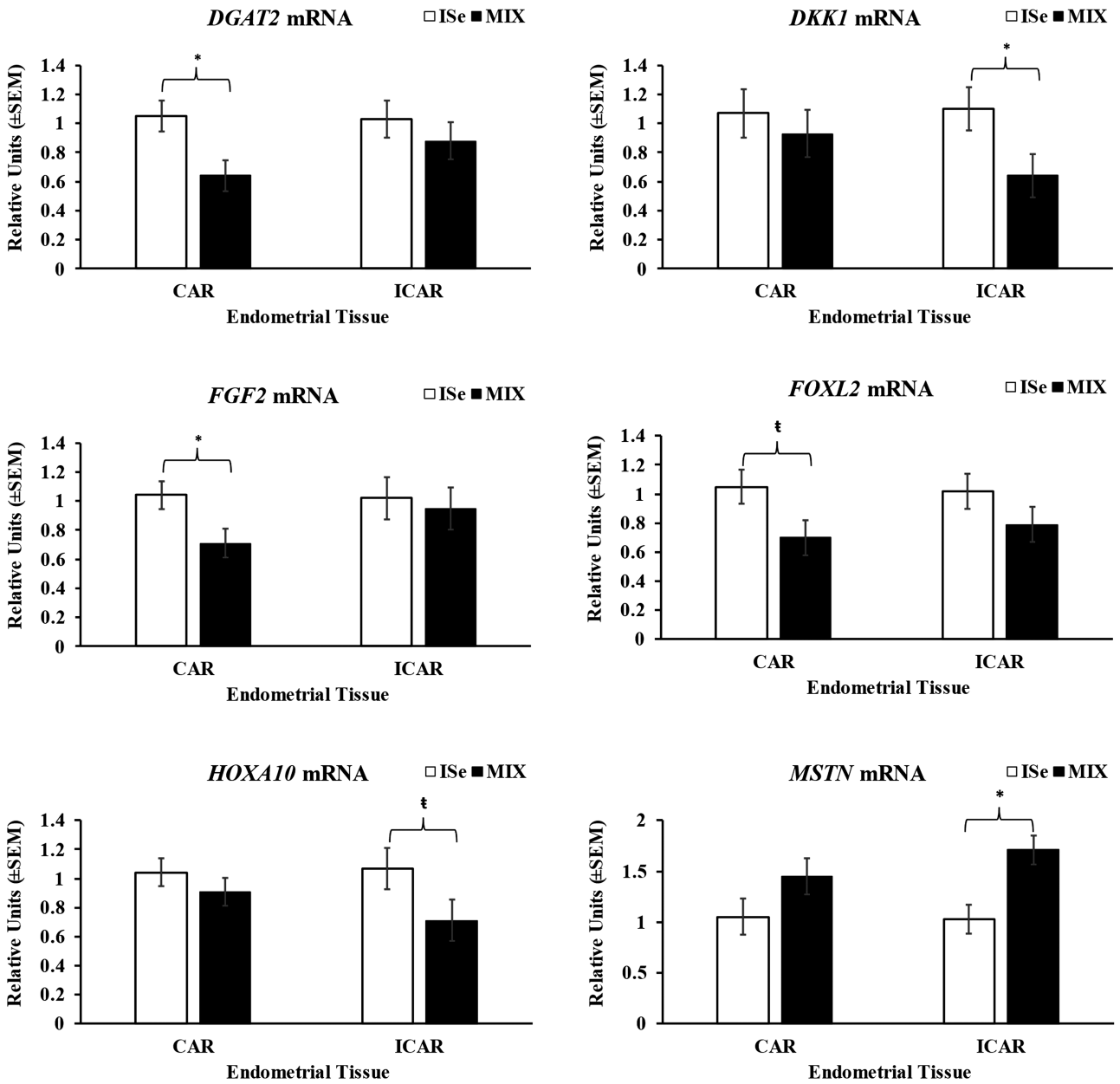

Figure 5. Effect of form of Se on the abundance of mRNA transcripts encoding progesterone-induced genes in the endometrium of heifers supplemented with vitamin–mineral mixes containing Se as ISe (sodium selenite; $n = 6$) or a 1:1 combination (MIX) of ISe and OSe (Sel-Plex; $n = 6$). *P*-values are associated with ANOVA. Significant differences at $P < 0.05$ are indicated by an asterisk and tendencies at $P \leq 0.1$ are indicated by ŧ.

al., 2008; Walker et al., 2010) and can induce ISG expression (Michalska et al., 2018). Similarly, IRF1 can regulate the expression of ISGs in response to type I and II interferons (Michalska et al., 2018). In the present study, qPCR results indicated MIX-supplemented heifers tended to have decreased abundance of *IRF1* and *IRF2* mRNAs compared with ISe heifers. Treating ovine luminal epithelia cells with IFNT-induced IRF1 expression within 1 h, with maximal expression observed at 3 h, followed by a steady decline through to 48 h (Stewart et al., 2001). This suggests that the unexpected MIX-induced decrease in the expression of this cohort of IFNT-induced transcripts may be reflecting a shift in the timing of MRP. It is possible that maximal expression of IFNT occurred earlier in the MIX-supplemented heifers, however, this possibility cannot be evaluated with the experimental design utilized in this trial. This would be consistent though with the postulated shift in the pattern of expression of *DGAT2* mRNA in MIX-supplemented heifers that is described above. Unfortunately, due to inconsistencies in the volume of uterine flush media retrieved during conceptus collection, quantification of concentrations of IFNT in the luminal fluid was not possible in this study.

During early pregnancy, OAS is upregulated and is involved in regulating the production of osteopontin (SPP1). In the present study, MIX heifers had an unexpected decrease in the relative abundance of mRNA encoding *OAS2* in CAR tissue compared with ISe heifers. An upregulation of SPP1 in pregnant animals promotes adhesion of the trophoblast to the endometrium, stimulates morphological changes in the trophoblast (Johnson et al., 2003), and regulates the immune response (Walker et al., 2010). Upregulation of these genes may be an important mechanism to enhance

Table 6. Real-time PCR[1] identification of selected steroidogenic enzymes and receptor genes from CAR and ICAR endometrium of heifers supplemented with 3 mg Se per d in vitamin–mineral mixes as sodium selenite (ISe, *n* = 6) or a 1:1 blend (MIX, *n* = 6) of ISe and OSe (SEL-PLEX)[2]

Gene	Gene name	CAR[3]				ICAR[3]			
		ISe	MIX	SEM	*P*-value	ISe	MIX	SEM	*P*-value
OXTR[4]	*Bos taurus oxytocin receptor*	1.54	1.40	0.49	0.85	2.2	2.03	0.56	0.91
ESR1	*Estrogen receptor 1*	1.07	0.79	0.13	0.16	1.04	0.86	0.11	0.26
PGR	*Nuclear progesterone receptor*	1.03^{x}	0.77^{y}	0.09	0.07	1.06	1.01	0.18	0.83
PGRMC1[4]	*Progesterone receptor membrane component 1*	1.02	1.10	0.08	0.50	1.05	0.98	0.11	0.85
PGRMC2	*Progesterone receptor membrane component 2*	1.05	1.02	0.12	0.85	1.03	1.03	0.09	0.97
EP1[4]	*Prostaglandin E receptor 1*	1.04	0.89	0.10	0.33	1.02	1.11	0.22	0.59
EP2	*Prostaglandin E receptor 2*	1.18	1.09	0.21	0.77	1.09	1.39	0.17	0.23
EP3[4]	*Prostaglandin E receptor 3*	1.20	0.75	0.21	0.17	1.34	1.07	0.39	0.42
EP4[5]	*Prostaglandin E receptor 4*	1.01	0.88	0.11	0.20	1.04	1.12	0.14	0.70
PAQR5[5]	*Progestin and adipoQ receptor family member 5 (mPR_{γ})*	1.31	1.24	0.25	0.69	1.07	1.15	0.17	0.76
PAQR7	*Progestin and adipoQ receptor family member 7 (mPR_{α})*	1.05	0.78	0.13	0.18	1.06^{x}	0.73^{y}	0.13	0.09
PAQR8	*Progestin and adipoQ receptor family member 8 (mPR_{β})*	1.07	0.89	0.14	0.37	1.06	1.03	0.15	0.91
PTGFR[6]	*Prostaglandin F receptor*	2.28	1.22	0.72	0.81	4.19	5.08	2.51	0.22
IFNAR1	*Bos taurus interferon alpha and beta receptor subunit 1*	1.05	0.86	0.10	0.23	1.04	0.94	0.13	0.61
IFNAR2[5]	*Bos taurus interferon alpha and beta receptor subunit 2*	1.02	0.89	0.09	0.31	1.02	0.91	0.11	0.48
PTGES	*Prostaglandin E synthase*	1.11	0.92	0.15	0.39	1.36	0.83	0.28	0.21
PTGS2[6]	*Prostaglandin-endoperoxide synthase 2*	1.05	0.91	0.19	0.40	1.20	1.56	0.23	0.25

[1]Data are expressed as a ratio of MIX relative to ISe expression.
[2]Selenium was supplemented at 35 ppm as either inorganic (ISe; sodium selenite) or a 1:1 combination (MIX) of ISe and OSe (SEL-PLEX). Selenium was supplemented to treatment groups ad libitum.
[3]Values are LS means and SEM.
[4]ICAR natural log transformed due to lack of normality.
[5]CAR natural log transformed due to lack of normality.
[6]CAR and ICAR natural log transformed due to lack of normality.
[a,b]Means within a row that lack a common superscript differ ($P < 0.05$).
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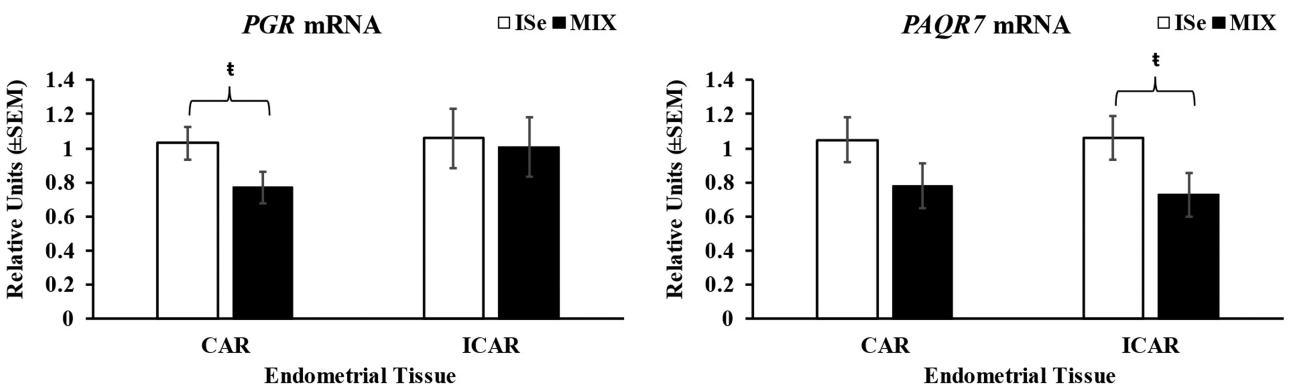

Figure 6. Effect of form of Se on the abundance of mRNA transcripts encoding *PGR* and *PAQR7* in the endometrium of heifers supplemented with vitamin–mineral mixes containing Se as ISe (sodium selenite; *n* = 6) or a 1:1 combination (MIX) of ISe and OSe (Sel-Plex; *n* = 6). *P*-values are associated with ANOVA. Tendencies at $P \leq 0.1$ are indicated by t.

the response to potential viral pathogens during the time of local immune suppression that occurs in response to the embryo (Walker et al., 2010). An upregulation of MX1 and MX2, both ISGs, supports this hypothesis (Hicks et al., 2003; Bauersachs et al., 2009). However, heifers supplemented with MIX also had decreased relative abundance of *MX1*, although a similar abundance of *MX2*, when compared with ISe-supplemented heifers in the present study. Additionally, MX genes induced by IFNT at preimplantation may play a role in pregnancy recognition, uterine reception, and/or conceptus attachment to the endometrium (Shirozu et al., 2015). Again, when coupled with the MIX-induced increase in length of the conceptus, the decrease in expression of *OAS2* and *MX1* transcripts in MIX-supplemented heifers appears to suggest a shift in the timing of MRP.

Additionally, supplementing heifers with MIX reduced the relative abundance of mRNA encoding radical S-adenosyl methionine domain containing 2 (*RSAD2*) in CAR samples. RSAD2 is produced during viral infection in response to interferons to limit viral replication and modulate adaptive

immunity (Helbig et al., 2005). Moreover, RSAD2 could act as an immunomodulatory factor preventing viral infection of the uterus during the critical stage of implantation (Mansouri-Attia et al., 2009). Interestingly, expression of *RSAD2* and *MX1* was not limited to the stroma or the glandular epithelium but was also detectable in the luminal epithelium at implantation (Mansouri-Attia et al., 2009).

Conclusion

Evidence from this study supports our hypothesis that the form of supplemental Se influences the expression of transcripts in the bovine endometrium at MRP and that MIX advances conceptus development compared with ISe. Results from qPCR indicated that heifers supplemented with MIX had decreased expression of several transcripts known to be induced by P4 and/or stimulated by interferons. Additionally, compared with ISe-supplemented heifers, MIX-supplemented heifers tended to have decreased abundance of mRNA encoding *PGR* and *PAQR7*, and the paradigm of loss of PR in uterine epithelia immediately before implantation occurs in both sheep and cattle, among other species. Similarly, MIX heifers tended to have decreased abundance of mRNA encoding *IRF1*, which is stimulated by IFNT and has reduced responsiveness to IFNT over time. Moreover, MIX-supplemented heifers had an increased abundance of mRNA encoding *MSTN*, which increases glucose secretion into histotroph, thus advancing conceptus development. The MIX-induced increase in conceptus length in MIX- vs. ISe-supplemented heifers is a salient finding of this experiment, with widespread implications for producers grazing cattle on Se-inadequate soils. Collectively, these results indicate more advanced conceptus development in cattle provided with MIX vs. ISe, with differences in endometrial gene expression at day 17 of pregnancy suggesting that the timing of MRP may also differ between ISe- and MIX-supplemented heifers.

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Conflict of interest statement

None of the authors of this article have any conflict of interest.

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