

Intraovarian WNT3A modulates estrogen-mediated estrus behavior in cattle

Bahaa H. Aloqaily,* Emily M. Ferranti,* Adam F. Summers,* Craig A. Gifford,[†] and Jennifer A. Hernandez Gifford*¹

*Department of Animal and Range Sciences, New Mexico State University, Las Cruces, NM 88003; and [†]Department of Extension Animal Sciences and Natural Resources, New Mexico State University, Las Cruces, NM 88003

© The Author(s) 2018. Published by Oxford University Press on behalf of the American Society of Animal Science.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com.

Transl. Anim. Sci. 2018.2:S19–S21
doi: 10.1093/tas/txy026

INTRODUCTION

The bovine estrous cycle consists of a long luteal phase maintained by progesterone, and a follicular phase encompassing less than 3 d of the entire cycle. During the follicular phase, follicle-stimulating hormone (FSH) upregulates estradiol (E₂) synthesis from granulosa cells of the developing follicles (Ireland et al., 1979; Ireland et al., 1980). Estradiol is an important regulator of folliculogenesis and fertility (Drummond and Findlay, 1999). Synthesis and secretion of E₂ relies on coordinated interplay between pituitary gonadotropins, luteinizing hormone (LH) and FSH, and intraovarian-signaling pathways. In the adult ovary, follicular development and steroid production is regulated in part by the wingless-type mouse mammary tumor virus integration-site (WNT) family of signaling molecules. Beta-catenin, a downstream molecule of canonical WNT signaling, is required for maximal FSH stimulation of the catalyzing cytochrome P450 enzyme aromatase, that converts androgens into E₂ (Parakh et al., 2006). Furthermore, granulosa cells lacking functional beta-catenin alleles have decreased aromatase mRNA expression and subsequent E₂ concentration (Hernandez Gifford et al., 2009). In large antral follicles of cattle with high intrafollicular E₂ concentration, a significant increase in beta-catenin protein abundance is

detected compared to follicles with lower E₂ concentration (Castañon et al., 2012). Additionally, aromatase gene expression and media E₂ concentration are muted in granulosa cells treated concurrently with FSH and WNT3A, indicating WNT regulation of FSH target genes (Stapp et al., 2014).

Collectively, results indicate that the WNT pathway modulates steroid production, but the effect of WNT signaling on follicular development, estrus activity, and ovulatory events is unclear. Therefore, the objective of the current study was to determine if transvaginal injection of WNT3A into the ovarian stroma at the time of follicle selection or recruitment impacts follicle development in beef heifers. Our working hypothesis is that exogenous WNT delivered during the follicular phase of the estrous cycle will mute E₂-mediated reproduction events.

MATERIALS AND METHODS

Animals

All animal procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee (#2016–033).

Fourteen Angus heifers (BW = 295 ± 50 kg) were housed at New Mexico State University beef facility and fed an ad-libitum total mixed ration. Heifer cyclicity was confirmed by serum progesterone concentration prior to initiation of the study. Four heifers did not respond to the estrous synchronization protocol and were excluded from the study.

¹Corresponding author: jgifford@nmsu.edu

Received March 16, 2018.

Accepted June 1, 2018.

Experimental Design and Treatments

Time of estrus was synchronized using GnRH (Factrel, 100 mg, i.m., Zoetis Inc., Kalamazoo, MI) on day 0 and intravaginal progesterone (Easi-breed CIDR, Zoetis Inc.) for 7 d followed by PGF2 α (Lutalyse 25 mg, i.m., Zoetis Inc.) on day of control internal drug release (CIDR) withdrawal. Heat patches (Estroject, Rockway Inc., Spring Valley, WI) were placed on the tail head, for visual detection of estrus performed every 12 h. An epidural was given (Lidocaine 2%, 5 mL/heifer, Vet one, Boise, ID), and utilizing an ultrasound (Aloka, SSD-500V) guided transvaginal probe treatments were delivered in a 1 mL volume over eight locations within the ovarian stroma. Treatment groups included vehicle-treated control (CTRL, $n = 3$); 500 ng/mL WNT3A (R&D systems, Minneapolis, MN) at time of CIDR withdrawal (WNT-REC, $n = 3$); or 500 ng/mL WNT3A 16 h following CIDR withdrawal (WNT-SEL, $n = 4$).

Twelve hours after observed heat, transrectal ultrasound of follicular dynamics was initiated. Thereafter, ultrasound evaluation was repeated after 6 h, then every 3 h until ovulation confirmed loss of the dominant follicle. Growing preovulatory follicle dimensions were recorded at each time point. Blood samples were collected (Corvac, Covidien, IA) every 6 h for serum E₂-concentration analysis.

Radioimmunoassay

Blood samples were centrifuged 1,500 gravity force for 15 min at 4 °C, serum was decanted and stored at -20 °C, until analysis. Estradiol concentrations were quantified using a commercially available RIA kit (MP Biomedicals, Solon, OH). Detection limit was 95% of maximum binding of the assay was 2 pg/mL. Intra-assay CV was 21% and interassay CV was 23%.

Statistical Analysis

All statistical analysis were performed using SAS (version 9.4; SAS Institute, Inc. Cary, NC). General linear models procedure was used. Estradiol concentrations and estrus behavior events were analyzed using ANOVA method and least squared means comparisons among treatments was performed only when a significant model ($P < 0.10$) was detected.

RESULTS AND DISCUSSION

WNT Modulates Estrogen-Mediated Events in Beef Heifers

Follicle-stimulating hormone upregulates granulosa cell expression of key steroidogenic enzymes necessary for E₂ production. Estradiol is important in many physiological processes especially those related to follicle development and fertility (Drummond and Findlay, 1999). Beta-catenin, a transcriptional cofactor in the canonical WNT-signaling pathway require for maximal stimulation of aromatase (Parakh et al., 2006) and subsequent E₂ synthesis (Hernandez Gifford et al., 2009). In bovine granulosa cells, increased beta-catenin abundancies were detected in follicles shown to be E₂ active (Castañon et al., 2012). However, WNT3A in combination with FSH, reduced aromatase gene expression, and E₂ synthesis in primary rat granulosa cells (Stapp et al., 2014). Interestingly, effect of beta-catenin on E₂ is dependent on the input signal to regulate beta-catenin. The ability of FSH and WNT to negatively regulate E₂ suggests these pathways could impact reproductive events in vivo.

To examine if WNT3A delivered into ovarian stroma reduces E₂ production during the follicular phase of the estrous cycle, heifers were treated with WNT3A at times corresponding to follicle recruitment, or follicle selection. Both WNT3A treatments delayed the onset of estrus ($P = 0.003$; Table 1), from 39 h in CTRL heifers compared to 93 h and 72 h for WNT-REC and after CIDR removal (WNT-SEL) treatment groups, respectively. In addition, time to ovulation was 66.3 h for CTRL compared to ($P = 0.008$) 128 h for WNT-REC and 101.25 h for WNT-SEL group ($P = 0.008$). However, time between standing heat and ovulation did not differ among treatment groups ($P > 0.10$; Table 1). Heat patches scores as a reflector of estrus intensity were lower ($P = 0.017$) for both WNT-REC and WNT-SEL injection treatments compared to CTRL.

Collectively, results suggest that WNT3A treatments altered reproductive behavior and follicular dynamics associated with estrus presumably by altering follicular E₂ synthesis. Interestingly, there was no significant difference in serum E₂ concentration ($P = 0.34$) between CTRL and heifers receiving WNT3A injection. It should be noted that ovarian injection with either CTRL or WNT treatment appeared to cause an accelerated decline serum E₂ (Figure 1) which might have masked the ability ($P = 0.34$) for WNT treatment to reduce serum E₂ at 6 h and 12 h after injection in the WNT-SEL group.

Table 1. Effect of intraovarian delivery of WNT3A on ovarian dynamics and estrus behavior

Item	Treatments ¹			SE	P value	Contrasts	
	CTRL	WNT-REC	WNT-SEL			CTRL vs. WNT ²	REC vs. SEL ³
Time to standing for heat, h	39 ^a	93 ^b	72 ^b	7.1	0.003	0.001	0.059
Time to ovulation, h	66.3 ^a	128 ^b	101.25 ^b	9.6	0.008	0.004	0.074
Time from heat to ovulation, h	27	35	29.25	4.1	>0.1	0.333	0.325
Heat patches score	2 ^a	1 ^b	1.25 ^b	0.2	0.017	0.006	0.35
Ovulatory follicle diameter, mm	13.2	9.6	11.3	0.9	0.063	0.034	0.20

¹Treatments: CTRL, ovaries injected with vehicle-treated control 16 h after CIDR withdrawal ($n = 3$); ovaries injected with 500 ng/mL WNT3A WNT-REC ($n = 3$); ovaries injected with 500 ng/mL WNT3A 16 h WNT-SEL ($n = 4$).

²Treatment means comparison between control and both WNT treatment groups = CTRL vs. WNT.

³Treatment means comparison of WNT-REC to WNT-SEL treatment groups = REC vs. SEL.

^{a,b}Means with different superscripts differ ($P < 0.05$) within a row.

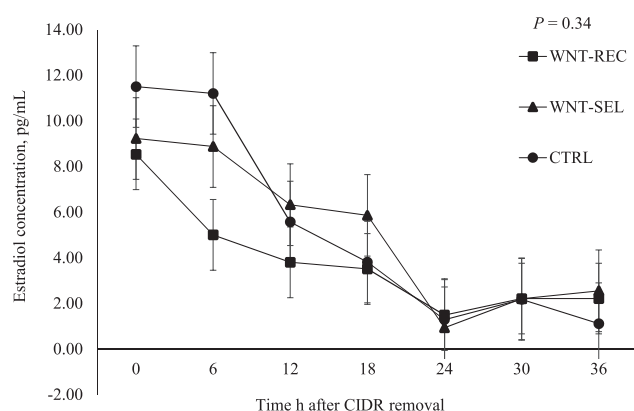


Figure 1. Serum estradiol concentration at 0, 6, 12, 18, 24, 30, and 36 h after CIDR withdrawal. In heifers treated with: 1) CTRL ($n = 3$); 2) WNT3A (500 ng/mL) WNT-REC ($n = 3$); and 3) WNT3A (500 ng/mL) 16 h WNT-SEL ($n = 4$).

Preovulatory follicles were tracked using transrectal ultrasound ovulation. Ovulatory follicle diameter collected at the time point prior to ovulation was evaluated among treatment groups. Both WNT-REC and WNT-SEL treatments had reduced ($P = 0.06$) ovulatory follicle diameter 9.6 mm and 11.3 mm, respectively, compared to CTRL (13.22 mm; Table 1). All heifers were observed for standing heat every 12 h for the next estrous cycle to determine if there were long-lasting effects of WNT3A on estrus behavior. All heifers regardless of treatment displayed estrus at the subsequent cycle. To determine if time to estrus was altered in subsequent cycles, all heifers were synchronized to estrus with the previously described protocol. Heifers were observed for estrus every 12 h, and there was no significant difference at time for estrus between treatments group ($P = 0.17$) compared to control.

CONCLUSION

Reproductive efficiency in cattle impacts production profitability. Estrogen concentrations

are critical for follicular maturation and control of estrus behavior. These results indicate that WNT3A administered during the follicular phase of the estrous cycle can impact reproductive events associated with ovarian estrogen production. The wingless-type mammary-integrated site-signaling pathway may be an important regulator of ovarian dynamics regulated by FSH in vivo.

LITERATURE CITED

- Castañón, B. I., A. D. Stapp, C. A. Gifford, L. J. Spicer, D. M. Hallford, and J. A. Hernandez Gifford. 2012. Follicle-stimulating hormone regulation of estradiol production: possible involvement of WNT2 and β -catenin in bovine granulosa cells. *J. Anim. Sci.* 90:3789–3797. doi:10.2527/jas.2011-4696
- Drummond, A. E., and J. K. Findlay. 1999. The role of estrogen in folliculogenesis. *Mol. Cell. Endocrinol.* 151:57–64. doi:10.1016/S0303-7207(99)00038-6
- Hernandez Gifford, J. A., M. E. Hunzicker-Dunn, and J. H. Nilson. 2009. Conditional deletion of beta-catenin mediated by amhr2cre in mice causes female infertility. *Biol. Reprod.* 80:1282–1292. doi:10.1095/biolreprod.108.072280
- Ireland, J. J., P. B. Coulson, and R. L. Murphree. 1979. Follicular development during four stages of the estrous cycle of beef cattle. *J. Anim. Sci.* 49:1261–1269. doi:10.2527/jas1980.504760x
- Ireland, J. J., R. L. Murphree, and P. B. Coulson. 1980. Accuracy of predicting stages of bovine estrous cycle by gross appearance of the corpus luteum. *J. Dairy Sci.* 63:155–160. doi:10.3168/jds.S0022-0302(80)82901-8
- Parakh, T. N., J. A. Hernandez, J. C. Grammer, J. Weck, M. Hunzicker-Dunn, A. J. Zeleznik, and J. H. Nilson. 2006. Follicle-stimulating hormone/camp regulation of aromatase gene expression requires beta-catenin. *Proc. Natl. Acad. Sci. U. S. A.* 103:12435–12440. doi:10.1073/pnas.0603006103
- Stapp, A. D., B. I. Gómez, C. A. Gifford, D. M. Hallford, and J. A. Hernandez Gifford. 2014. Canonical WNT signaling inhibits follicle stimulating hormone mediated steroidogenesis in primary cultures of rat granulosa cells. *Plos One.* 9:e86432. doi:10.1371/journal.pone.0086432