

# Streams of a career in research: “It was a wonderful journey, this career of mine”

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## STREAMS OF A CAREER

The first question a reader might ask is, “what does he mean by streams of research?” Well, a research stream may be defined as a series of related experiments or articles on one topic, each progressing to dig deeper, especially to discover new or unique information or to reach a new or more profound understanding: the best stream is programmatic and systematic, progressing from a hypothesis or conceptual mechanism, to fundamental, quantitative research. Ultimately, this stream may lead to an application. A vibrant stream does not end [adapted from Peng (2010) and the Cambridge Academic Content Dictionary (2019)]. I hope this is made clear to the reader by the end of this monograph.

This journey is based on the premise/assumption that by understanding the variation in biological processes, we might be able to discover the underlying biological mechanisms that will allow us to improve the efficiency and sustainability of the biological system that we are studying, especially for economically important species. The major streams of research in my career included 1) physiological mechanism(s) whereby the biostimulatory effect of bulls accelerates resumption of cycling activity and improves fertility in heifers and in primiparous, postpartum, suckled beef cows; 2) factors that influence fertility at puberty in heifers and ewe lambs, and at the resumption of ovulatory cycles in postpartum, suckled beef

cows; 3) fertility of heifers and postpartum cows exposed to bulls during estrous synchronization protocols that incorporate a controlled internal drug-releasing device; and, 4) developing the use of nuclear magnetic resonant spectroscopy of small molecular weight metabolites to identify critical interactions of metabolites and metabolic hormones involved with reproductive endocrine function, disease, and behavior of domestic and wild ruminant species. There were many other “streamlets” in my career that were exceptionally interesting and productive; however, I do not have the space to discuss these topics in this article.

The following narrative represents the chronicles of “STREAM 1,” namely, the biostimulatory effect of bulls on postpartum, primiparous, anovular, suckled beef cows. The long-term goal of “Stream 1” was to determine the physiological mechanism(s) and pheromonal pathways by which the biostimulatory effect of bulls accelerates the reproductive neuroendocrine–endocrine cascade that culminates in resumption of ovulatory cycles in postpartum, anovulatory, suckled cows. My hope was to develop technologies based upon the fundamental mechanism(s) involved with the biostimulatory effect of bulls that would increase reproductive efficiency of cow–calf operations by providing management strategies that were low cost, were time and labor saving, yielded consistent results and returns, and were easily applicable, socially acceptable, and sustainable.

## STREAM 1: PUTTING IN (1974 TO 1979)

As a young M.S. student in 1974, under the supervision of Dr. E. K. Inskeep, I got my

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introduction to phenomenon of “biostimulation,” although this word had yet to be coined in the literature. The term “biostimulation” originally appeared in the literature in 1983 to refer to any stimulatory effect of a male on estrus and ovulation in females through genital stimulation, priming pheromones, or other less defined external cues. However, there are cases where females may have a biostimulatory effect on males or on other conspecific females; thus, this term is not mutually exclusive for a given sex in mammals (Chenoweth, 1983). One could say that most of my career centered around this “biostimulatory effect” of males on anestrous or anovular females.

By this time, it was known that the presence of an adult male reduced the age at puberty in female mice and gilts, and hastened the onset of the breeding season in sheep and goats (for review, see Rekwot et al., 2001). So, we (actually, Dr. Inskeep) asked the question, does the presence of a mature bull for 21 d accelerate age at puberty in beef heifers? The answer to this question was NO, the presence of a bull, under these conditions, did not influence age at puberty in beef heifers (Berardinelli et al., 1978). We then asked, if we “primed” heifers with progesterone (P4) and increased the exposure period to 30 d, would combining this treatment with the presence of bulls accelerate age at puberty in prepubertal heifers? Again, the answer was NO; this combination did not appear to accelerate age at puberty in heifers (Berardinelli et al., 1978). Thus, my first experience with biostimulation was certainly not positive. But I had learned much about how to do science, gained vital experience in learning field and laboratory techniques, understanding the value of statistics, and learning how to write in a scientific manner during this 5-yr period at West Virginia University (WVU). I was learning how to paddle a canoe.

### **STREAM 1: INTO THE MAIN CHANNEL (1981 TO 2001)**

I began my academic career by joining the faculty of Montana State University in the Department of Animal and Range Sciences in 1981 as an assistant professor. This required me to develop a modern laboratory (for the time) from scratch and establish a research program in reproductive physiology and endocrinology. These were no easy tasks at the time. However, I had a very good mentor and collaborator in Dr. Peter Burfening, whom I thank for all his support and encouragement during my career. As important as he was in shaping my career were the members of the Western Section

Technical Committee, W112, especially Drs. Ron Randall, Bob Short, Bob Staigmiller, Bob Bellows, Terry Nett, and Dennis Hallford. And, there are many more collaborators on this committee that I lack the space to mention but I would like to thank. They were a tremendous resource and an invaluable network over the past 37 years.

So, my “paddling” continued with one of the objectives of the W112 at the time, which was to determine those factors that limit reproductive performance of beef heifers and postpartum, anestrous cows. My first attempt at WVU to use mature bulls to accelerate the onset of puberty in heifers proved disheartening; however, I reasoned that those negative results might have been caused by small numbers of heifers and limited exposure periods. Perhaps, heifers needed to be exposed to bulls for longer than 30 d. Dr. Mark Roberson, then an MS student, and I tested this hypothesis by starting bull exposure at 200 d of age on 100 prepubertal heifers. Again, we found that bull exposure of beef heifers for 152 d did not accelerate age at puberty (Roberson et al., 1987). We concluded that bull exposure of prepubertal beef heifers to long-term exposure of bulls did not influence the occurrence of puberty under these conditions. Another “tip of the canoe.” However, as we will see in the discussion of postpartum, anovular cows, conditions matter!

While I was working on the effect of bull on heifers, I read an article from Nebraska indicating that resumption of estrous cycles was advanced by exposing mature, multiparous cows to bulls during the early postpartum period (Zalesky et al., 1984). Ah, here is a case where a clear biostimulatory effect occurs in cattle. As I dug into the literature, I failed to find a single article regarding effects of bull exposure on primiparous suckled, anovular beef cows, and Zalesky et al. (1984) made no mention of the physiological mechanisms by which bulls might influence postpartum reproductive function of suckled cows. Luckily, and thanks to Dr. Richard (Butch) Whitman, there was a management change in 1985 involving first-calf cows in our herd. They would now be housed and maintained on the farm in Bozeman. This was vitally important for it allowed me direct and easy access to this type of cow, and with some effort, I could manipulate the facilities to isolate groups of cows and bulls from one another. In hindsight, it was like I could stop “paddling” and use a motor on this journey.

Our first experiment involved exposing first-calf, suckled cows to bulls continuously, starting 3 d after calving to determine whether this type of exposure would alter postpartum interval to estrus,

and patterns of luteinizing hormone (LH) concentrations. More importantly, we thought to get a glimpse of a mechanism by taking serial, 15-min blood samples for 6 h at weekly intervals starting 10 d after initial exposure of cows to bulls for assay of LH. Our hypothesis was that characteristics of pulsatile patterns of LH would increase sooner in cows exposed to bulls (BE) than in cows not exposed to bulls (NE). Results of two trials clearly demonstrated that the presence of bulls accelerated resumption of ovulatory cycles in primiparous, suckled beef cows. However, characteristics of LH patterns in weekly sample did not indicate that the presence of bulls altered temporal pattern of LH (Custer et al., 1990). This result did not seem to fit the consensus hypothesis that in order to accelerate resumption of ovulatory cycles in anovular females, any stimulus(i) should affect the hypothalamic–pituitary axis to increase GnRH and in turn increase pulse frequency of LH, which stimulates final stages of follicular development, estradiol increase, and ovulation. Perhaps the timing of sampling was insufficient to determine if the presence of bulls altered this axis and LH patterns.

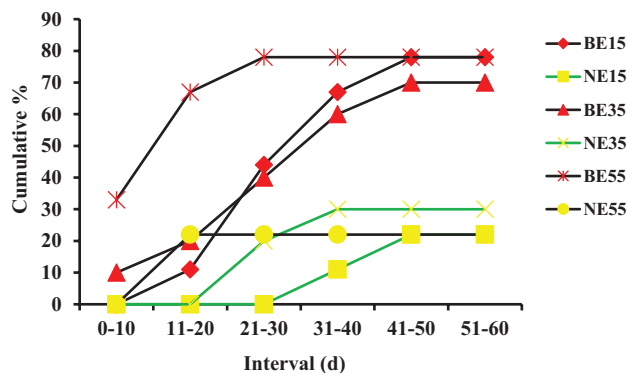
At this point, we thought two important issues had to be addressed to gain some insight into this physiological mechanism. The first was whether cows needed to be exposed continuously to bulls “soon” after calving to obtain a biostimulatory response, and the second was whether continuous, 24-h exposure, was required to elicit a biostimulatory response. Dr. Dave Fernandez was a graduate student of mine at the time that tackled these difficult issues. First, we exposed of primiparous, suckled beef cows to mature bulls in the first 30 days after calving (BE/NE), exposed them beginning 30 d after calving (NE/BE), or exposed them continuously starting 3 d after calving (BE). We found that postpartum intervals to resumption of ovarian cyclic activity did not differ among BE, BE/NE, and NE/BE cows, but they were 15.4 d shorter than for NE cows (Fernandez et al., 1993). We now knew that we could obtain a biostimulatory effect of bulls by exposing cows continuously 30 d after calving that was the same as continuous exposure beginning 3 d after calving.

We then exposed cows to a bull initially on day 30 after calving (NEBE), exposed cows to a bull for 2 h every third day beginning on day 30 after calving (intermittent exposure; BEI), or did not expose cows to a bull (NE). More importantly, this design gave us the opportunity to collect serial blood samples from cows to evaluate temporal LH patterns acutely and chronically after exposing cows to bull. Blood samples were obtained over a 6-h period at

15-min intervals every third day for 18 d. Sampling from BEI cows began 2 h before introduction of a bull every other day. We found that mean LH and LH pulse frequency increased within the 6-h sampling period in both NEBE and BEI cows to bulls compared to NE cows. Surprisingly, even though LH pulse frequency increased with intermittent 2-h exposure every third day, these cows did not respond to the biostimulatory effect, i.e., interval to resumption of ovulatory activity did not occur any sooner than that in NE cows.

On the basis of these data and the hypothesis that increased LH secretion plays a significant role in the resumption of ovarian cycling activity, one might conclude that bulls effect a reduction in postpartum interval to ovulation by increasing LH secretion acutely and chronically after exposure. However, cows that were intermittently exposed to bulls did not exhibit estrus and ovulation any sooner than cows isolated from bulls. One interpretation of these results is that immediate exposure to bulls induces a pheromonally activated trigger (signaling type) that induces a hypothalamic release of GnRH and subsequent acute release of LH, specifically an increase in pulse frequency. However, this type of stimulation (conditions) does not result in the induction of ovarian cycling activity. We postulated that there must either be exteroceptive cues other than pheromones or that there is some type of “re-enforcement mechanism” associated directly with the physical presence of the bull involved in the biostimulatory effect of bulls on postpartum cows (Fernandez et al., 1996). This was a dilemma that needed attention in order to understand this mechanism.

Before we tackled this dilemma and to gain a more profound understanding of the biostimulatory effect of bulls it was vital to precisely nail down the temporal development of the response of postpartum anovular cows to the biostimulatory effect of bulls. The question to be answered was are primiparous, anovular, suckled cows more sensitive to the biostimulatory effect of bulls when exposure to bulls occurs at progressively longer intervals after calving? To answer this question, we exposed primiparous, anovular, suckled cows to bulls at 15, 35, or 55 d after calving. Evaluation of the cumulative 10-d distribution of percentages of cows that resumed ovulatory activity among cows exposed to bulls beginning 15, 35, and 55 d after calving indicated that cows become responsive to the biostimulatory effect of bulls about 35 to 40 d after calving and sensitivity increases as time after calving increases (Fig. 1; Berardinelli and Joshi, 2005).

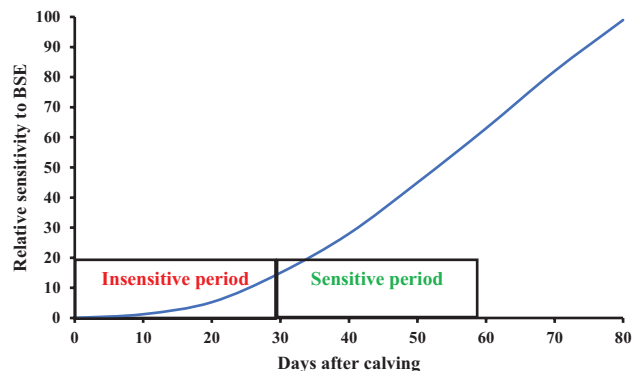


**Figure 1.** Cumulative percentages of primiparous, suckled beef cows exposed to presence of a bull (BE) or not exposed to a bull (NE) on either days 15, 35, or 55 after calving that resumed ovarian cycling activity. Interaction  $\chi^2 = 18.6$ ,  $df = 10$ ;  $P < 0.05$ .

This was an important finding that impacts the effectiveness of the biostimulatory effect of bulls during early postpartum anestrus of cows. We would have recognized this earlier if I would have examined the differential in intervals to resumption of ovulatory activity between cows exposed to bulls starting 3 d after calving compared to cows exposed on day 30 after calving (Fernandez et al., 1996). The differential was much shorter for cows exposed beginning on day 30 after calving than for cows exposed 3 d after calving! Nevertheless, the question then became what is it about those first 35 d after calving that dampens or attenuates the biostimulatory effect of bulls?

The answer to this question may be the following. This corresponds to the period during which inhibitory influences on LH secretion, such as increased sensitivity to the negative feedback of estradiol, the maternal cow–calf bond, and lactational stimuli are still very high. Possible explanations for this observation are either there is no pheromonal mechanism in cows during this period to respond to bulls and they developed them especially for this time in their reproductive life cycle (not likely), or the pheromonal mechanism is present but the negative effects that suppress LH release have the same inhibitory effect on the pheromonal system that mediates the biostimulatory effects of bulls (highly likely). If the latter is the case, then the apparent “insensitive period” is related to the intensity of stimulation. Increasing the “intensity” (dose or duration of exposure) should induce a response to the biostimulatory effect of bulls. If the former is true, then no level of intensity of bull biostimulation will evoke a response. Nevertheless, cows begin to become responsive to the biostimulatory effect of bulls about 35 to 40 d after calving, and it appears that sensitivity increases as time after calving increases (Berardinelli and Joshi, 2005). An illustration of this conceptualization is presented in Fig. 2.

Translate basic science to industry innovation

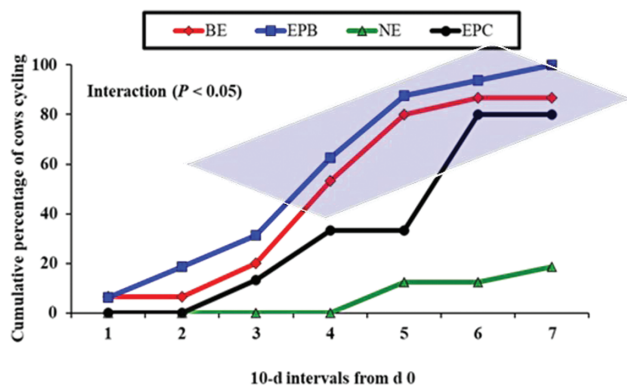


**Figure 2.** Relative hypothetical sensitivity of primiparous, anovular, suckled beef cows to the biostimulatory effect (BSE) of bulls after calving (day 0).

Please note that we think that insensitive to sensitive periods can slide left or right depending on the influences of other major factors that affect the length of postpartum anestrus in suckled cows.

Around 2000, it became apparent to my laboratory that a critical feature of the biostimulatory effect of bulls was missing, that was, what might it be and what is its source? We and most investigators at the time assumed or speculated that bulls produce a primer pheromone that acts via an olfactory pathway to evoke this response (for review, see Rekwot et al., 2001). Thus, we investigated the hypothesis that the biostimulatory effect of bulls is mediated by exteroceptive stimuli of bulls in a series of three experiments. The first experiment tested the hypothesis that exposing postpartum, anovular suckled cows to excretory products of bull for 12 h daily would evoke the same biostimulatory response as continuously exposing cows to the physical presence of bulls. Indeed, we found that interval to resumption of ovulatory activity and the proportions of cows that resumed ovulatory activity were shorter and greater, respectively, for cows exposed to a bull continuously or cows exposed to the excretory products of bulls for 12 h daily compared with those metrics of cows not exposed to a bull or excretory products of bulls. More importantly, the temporal responses for the proportions of cows cycling at 10-d intervals after exposure to a bull or the excretory products of bulls were identical (Fig. 3; Berardinelli and Joshi, 2005).

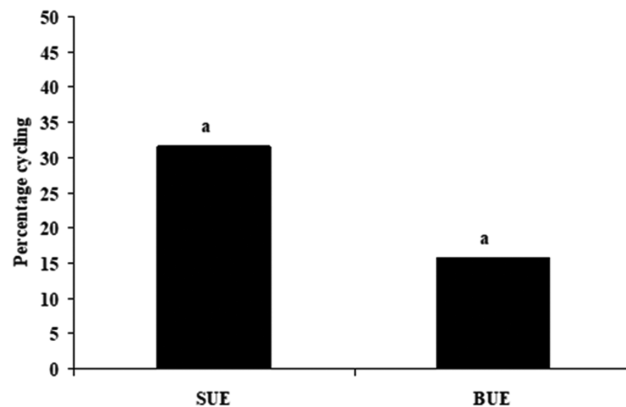
These data clearly indicate that excretory products (urine, feces, or mucus/saliva) of bulls hastened the resumption of ovulatory activity in postpartum, anovular, suckled cows. Therefore, the biostimulatory mechanism appears to be mediated by a pheromone(s) present in their excretory products and does not appear to be reinforced by the physical presence of bulls. This was an extraordinary



**Figure 3.** Cumulative percentages of first-calf restricted suckled beef cows exposed continuously to presence of a bull (BE), exposed to excretory products of bulls (EPB), not exposed to a bull (NE), or exposed to excretory products of cows (EPC) that resumed ovarian cycling activity in 10-d intervals from day 0 to end of the experiment. The blue-shaded area represents points that do not differ ( $P > 0.10$ ) among BE, EPB, and EPC cows.

discovery at the time. But, could we nail down a specific excretory product that would be a major carrier for such a pheromone?

Perhaps we could as [Baruah and Kanchev \(1993\)](#) reported that bull urine sprayed into the nasal passages of dairy cows 7 d after calving increased systemic LH and follicle stimulating hormone concentrations within 70 min of exposure, and we knew that other reproductively active pheromones in mammals are carried in urine. We asked the question: does exposing cows to mature bull urine mimic the biostimulatory effect of bulls to reduce the postpartum interval to ovulatory activity in primiparous, anovular suckled cows? In an elegant experiment by [Tauck et al. \(2006\)](#), we tested the hypothesis that continuous exposure (24 h) of cows to bull urine would accelerate resumption of ovulatory activity. Urine from bulls and steers was delivered continuously to cows by means of an ingenious, controlled urine deliver device. We found that interval from urine exposure and proportions of cows that resumed ovulatory activity did not differ between BUE- and SUE-exposed cows after 64 d of exposure ([Fig. 4](#)). This was a disconcerting result. However, we reasoned that perhaps 24-h exposure of cows to bull urine, under these conditions (again, conditions) in some manner, actually “desensitized,” downregulated, or otherwise stressed the physiological system(s) that sense and perceive a urinary pheromone by cows. To test whether this might be the case, we exposed primiparous suckled cows to mature bull urine or saline by constant drip for 12-h daily onto straw-bedding, and then placed cows into these areas for 12-h daily. Again, 12-h exposure to straw-bedding containing bull urine did not affect interval to resumption of ovulatory



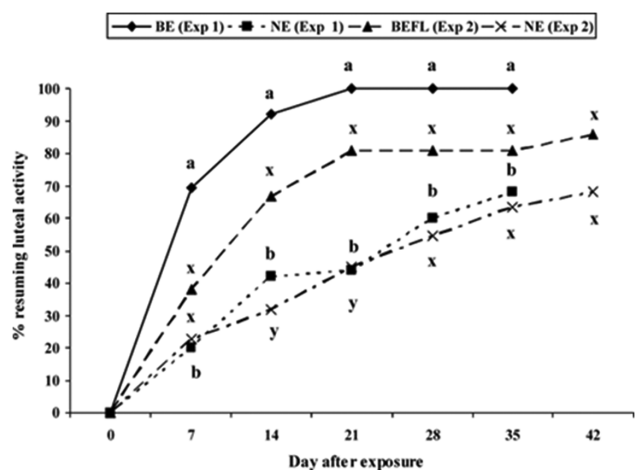
**Figure 4.** Percentages of first-calf suckled cows exposed to mature bull urine (BUE) or exposed to steer urine (SUE) that resumed ovarian cycling activity by the beginning of the estrus synchronization protocol. Bars with common letters do not differ,  $P = 0.25$ ,  $\chi^2 = 1.3$ ,  $df = 1$ .

activity ([Berardinelli et al., 2010](#)). We concluded that continuous or 12-h exposure of postpartum, anovular, suckled cows to bull urine does not allow for a pheromonally induced activation of the hypothalamus–pituitary–ovarian (HPO) axis to stimulate the cascade resulting in resumption of ovulatory activity.

However, these results do not preclude the possibility that bull urine contains a biostimulatory pheromone(s), but may indicate that the “mode” (frequency, amplitude, duration) of pheromonal stimulation may be an important factor that determines the biostimulatory effect of bulls on resumption of luteal activity in postpartum, suckled beef cows. Too much for too long each day may “desensitize” or overly stress the HPO axis, whereas too little or too short a duration of exposure may be insufficient to be recognized as stimuli to generate a response by the HPO axis of primiparous, anovular, suckled cows.

That this might be the case can be inferred from an experiment involving fenceline contact between bulls and cows. We found that exposing cows to direct fenceline contact (BEFL) with bulls penned within their pen accelerated resumption of ovulatory activity compared with cows not exposed to bulls (NE). However, the biostimulatory response of cows exposed to fenceline contact was not as dramatic as that for cows exposed to the physical presence of bulls ([Fig. 5](#); [Berardinelli and Tauck, 2007](#)).

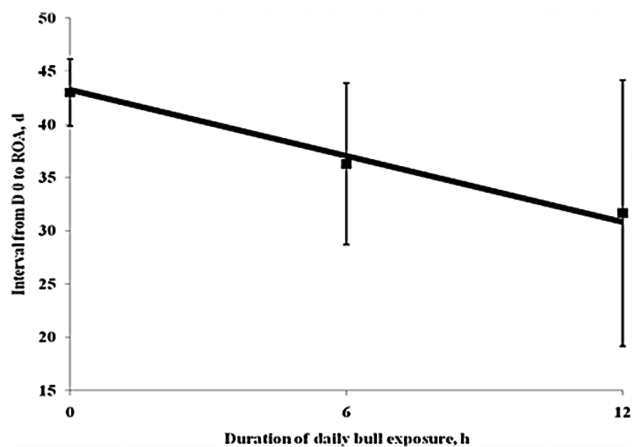
Although these data indicate qualitatively that intensity of the biostimulatory effect of bulls is an important factor to consider in this effect, they did not yield insight regarding the quantity and duration of exposure necessary to evoke a response. As I stated previously, these are critical variables that we had yet to measure. Nevertheless, we think that



**Figure 5.** Cumulative frequency distribution of the percentages of primiparous, anovular cows exposed to close physical contact with mature bulls (BE) or not exposed to bulls (NE) for 35 d and exposed to fenceline contact with bulls (BEFL) or not exposed to bulls (NE) for 42 d.

collectively, these data indicated that there is a minimum and maximum intensity to the pheromonally mediated effect of bulls, if the pheromone is carried by urine.

The next critical question that we asked was does the response of cows to the biostimulatory effect of bulls involve a duration of exposure component? [Fernandez et al. \(1996\)](#) reported that interval from calving to resumption of ovulatory activity was not accelerated in cows exposed to bulls for 2 h every third day for 18 d beginning 33 d after calving. However, length of postpartum anestrus was reduced in cows exposed to the excretory products of bulls for 12 h daily ([Berardinelli and Joshi, 2005](#)). With these results in mind, we tested the hypothesis that duration of bull exposure and interval to resumption of ovulatory activity in primiparous, anovular, suckled cows exposed to bulls for 0, 6, or 12 h daily would progressively accelerate resumption of ovulatory activity in anovular, suckled primiparous cows. Indeed, the results of this experiment showed that the number of hours of daily bull exposure required to accelerate resumption of ovulatory in postpartum, anovular, suckled cows decreases in a linear manner ([Tauck et al., 2010a; Fig. 6](#)). Thus, the duration of bull-pheromone stimuli that cows perceive each day is related to how soon after exposure primiparous, postpartum, anestrus, suckled cows respond to this stimulus and undergo the physiological changes necessary to resume ovulatory activity. The dose-dependent manner by which pheromones produced by bulls accelerated resumption of ovulatory activity in postpartum, anovular, suckled cows may explain disparate reports in the literature concerning fenceline contact and intermittent exposure of cows to bulls.



**Figure 6.** Linear regression of interval from the start of bull exposure (D 0) to resumption of ovulatory activity on hours of daily exposure for primiparous, postpartum, anovular, suckled cows exposed to bulls for 0, 6, or 12 h. Y-intercept ( $b_0$ ) = 42.2 d; slope ( $b_1$ ) = 1.01 d/h ( $P < 0.05$ );  $R^2 = 0.18$ .

Integrating the results of our “stream of research” to this point, we developed the concept that cows respond to bull-pheromonal “stimulation and relaxation cycles” in a dose-dependent manner. This type of cyclic presentation of stimuli may be a critical component of the pheromonal mechanisms by which the biostimulatory effect of bulls accelerates resumption of ovulatory activity. However, it appeared to us, that in some way, there was some type of reinforcement mechanism or agent related to dosage, intensity, and duration of the mechanism involved a pheromonally mediated biostimulatory effect of bull.

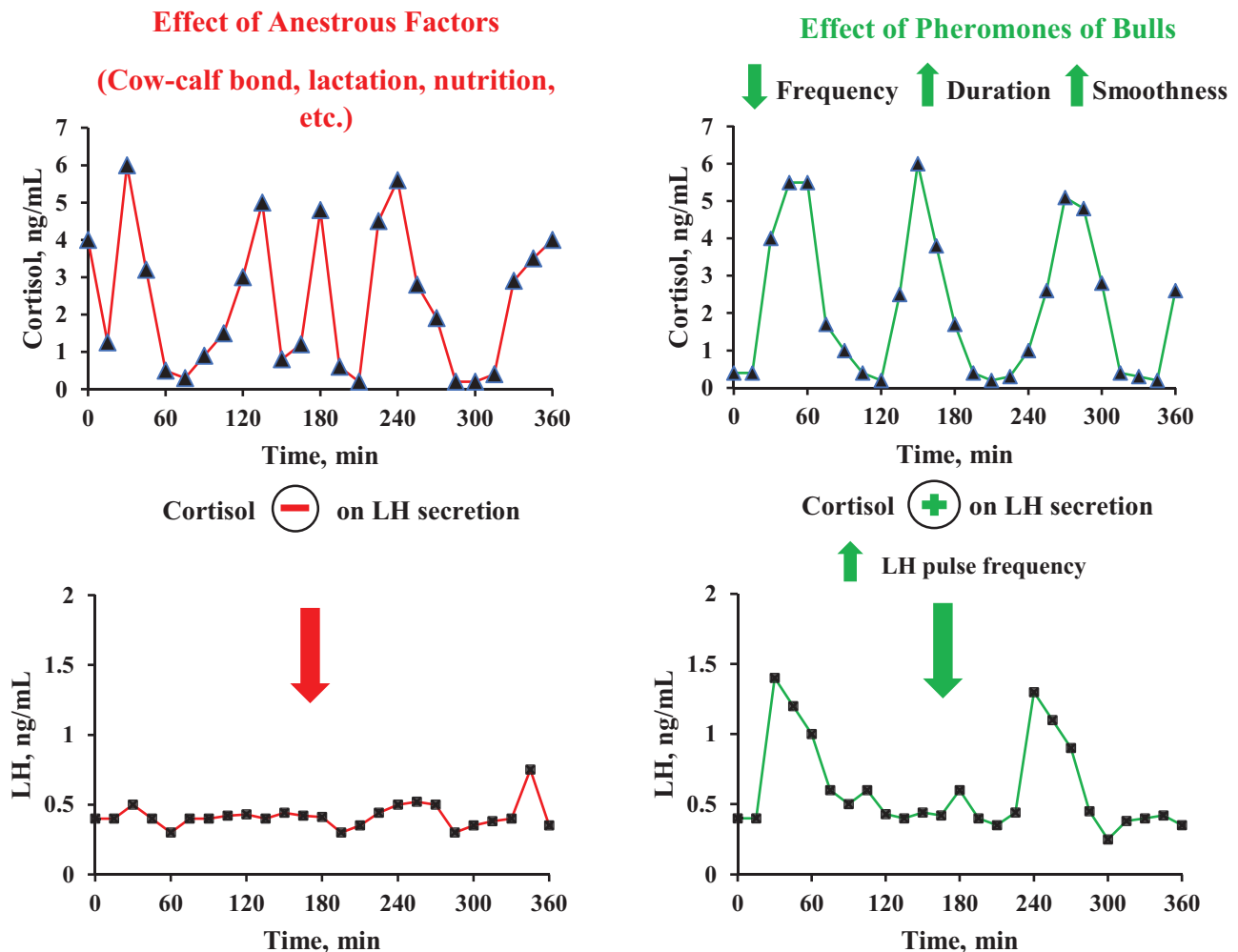
This idea led us to the following question: what physiological factor(s) might act as a “triggering” and (or) “re-enforcing” agent for the putative pheromonal mechanism to stimulate resumption of ovulatory activity? In rodents, there appeared to be a clear functional relationship between the hypothalamic–pituitary–adrenal (HPA) axis and induced pheromonal activation or inhibition of female reproductive events ([Mora and Sánchez-Criado, 2004](#)). Might there be the same relationship in the bovine? To answer this question, we evaluated cortisol concentrations in blood samples obtained at 3-d intervals from cows exposed continuously to bull urine (BUE) and cows exposed to the physical presence of bulls (BE) starting 35 d after calving ([Tauck et al., 2007](#)). Remember, interval to resumption of ovulatory activity for BUE cows did not differ from that in SUE cows, but BE cows resumed cycling much sooner than NE cows ([Tauck et al., 2007](#)). Surprisingly, systemic cortisol concentrations in BE cows increased significantly within 9 d after exposure, and the difference in cortisol concentrations was maintained throughout the exposure

period compared to those of NE cows, and all BE cows resumed cycling activity! In contrast to these results, cortisol concentrations in BUE cows clearly follow the same temporal pattern as that of SUE cows over the exposure period, and BUE cows had the same interval to resumption of ovulatory activity as SUE cows. We suggested that these data indicated a strong possibility that activation of the HPA axis might be intimately, but subtly, involved with the pheromonally mediated biostimulatory effect of bull (Tauck et al., 2007).

To gain insight into this possibility, we evaluated characteristics of temporal patterns of cortisol and LH in postpartum, anovular, suckled beef cows after acute exposure to bulls. Our hypothesis was that acute exposure of cows to bulls for 5 h daily over a 9-d period would increase cortisol concentrations, and in turn, increase mean and frequency of LH concentrations like that which would trigger the neuroendocrine–endocrine ovulatory cascade. We reported that exposing postpartum, anovular, suckled cows to bull in this manner altered

characteristics of temporal patterns of both LH and cortisol by increasing LH pulse frequency and decreasing cortisol pulse frequency. Interestingly, in cows exposed to bulls, as amplitude and frequency of cortisol pulses decreased, amplitudes of LH pulses increased, and frequency of LH pulses tended to increase (Tauck et al., 2010b). Thus, the physiological mechanism of the biostimulatory effect of bulls may initially involve modification of the HPA axis, and these changes may facilitate activation of the HPO axis and resumption of ovulatory cycles in postpartum, anovular, suckled cows (Tauck, 2008). Our interpretations of these results are graphically presented in Fig. 7.

Finally, in this stream, there is “the rest of the story.” For resumption of ovulatory activity to occur in postpartum, anovular suckled cows, changes in LH pulse frequency related to ovarian follicular development, maturation of dominant follicles (DFs), and ovulation are required. Therefore, it was reasonable to postulate that as the physical presence of bulls to cows increases pulse frequency of

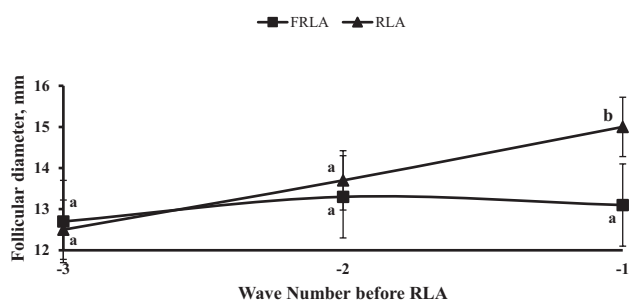


**Figure 7.** Graphic representation of the effect of factors that influence length of postpartum anestrus and the biostimulatory effect of bulls on the hypothalamic–pituitary–adrenal axis of postpartum, anovulatory, suckled beef cows.

LH in postpartum cows, the biostimulatory effect of bulls may influence follicular wave dynamics of exposed cows. Indeed, in the studies by Wilkinson (2009), it was reported that exposing cows to bulls altered follicular growth and developmental patterns by decreasing the number of follicular waves and increasing mean follicle diameter before resumption of ovulatory activity (Fig. 8). We interpreted these results to mean that the biostimulatory effect of the bull reduces the postpartum interval of anovulation by causing cows to produce larger DFs that are capable of ovulating sooner than cows not exposed to bulls. It is possible that a component of the biostimulatory mechanism for accelerating the resumption of ovulatory activity in postpartum, anovular, suckled cows intimately involves activation of the HPO axis by producing larger DFs capable of producing LH receptors allowing them to secure LH dependence sooner and ovulate earlier under appropriate endocrine signals.

### STREAMS 1: PADDLING TO THE BANK

So where does this leave us? After 33 yr in this “stream,” I concluded that the response of anovular, primiparous cows to the biostimulatory effect of bulls may be depended on the intensity of exposure (frequency of exposure, duration of exposure, and quantity of stimuli) of pheromonal stimuli produced by bulls. On the basis of the results and interpretation from the literature in other species, and the results of this stream of research, it is obvious that the physiological mechanisms responsible for regulating this process are extraordinarily complex and very much dependent on conditions that influence the mode of transmission of pheromonal signals between bulls and cows. In the following narrative, I will try to summarize my proposed model of this complex



**Figure 8.** Least squares means for the maximum dominant follicular (MDF) diameter in mm, three follicular waves before the resumption of luteal activity (RLA) for cows exposed to bulls that RLA during the 45-d exposure period and those cows exposed to bulls that failed to RLA (FRLA) during the exposure period and were assigned the end of the exposure period as their RLA date. The vertical bars represent the pooled SEM. Means among time points lacking a common superscript are different ( $P < 0.05$ ).

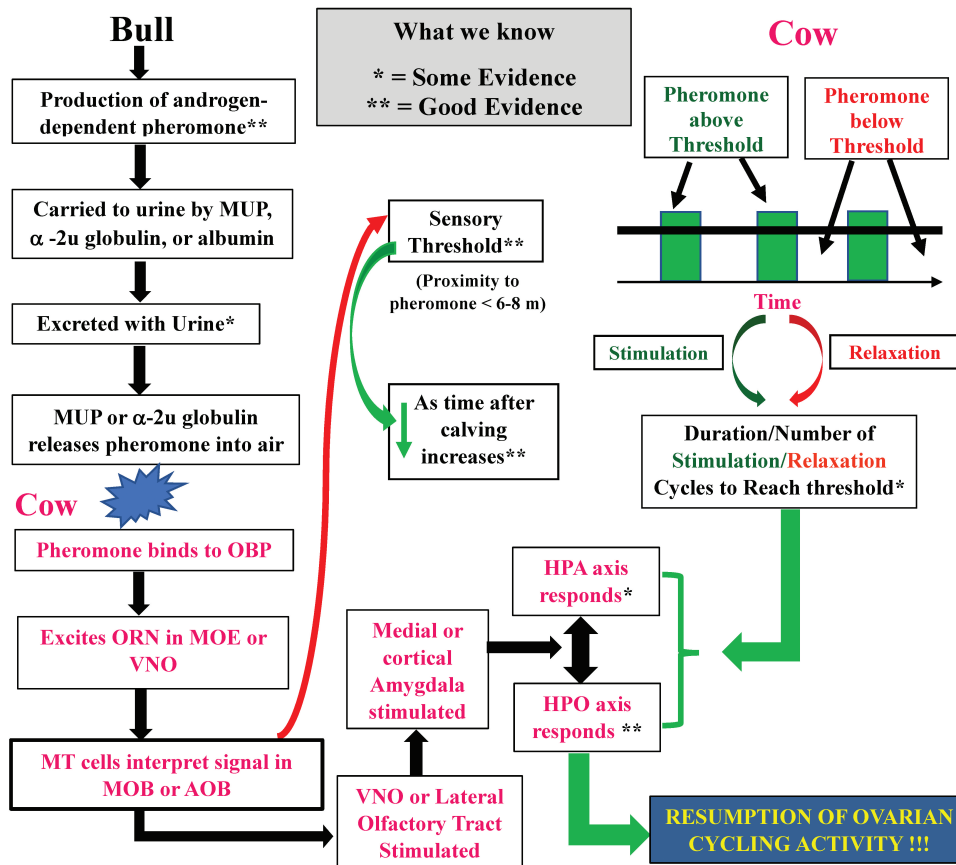
mechanism with the following illustration (Fig. 9) and interpretation.

Bulls produce an androgen-dependent pheromone that is carried from the blood to the urine by major urinary protein (MUP),  $\alpha$ -2u globulin, albumin, or a combination thereof. This pheromone is excreted in urine, feces, cutaneous secretions, or a combination of these excretory products. Alpha-2u globulin, MUP, or albumin releases the androgen-dependent pheromone into the environment after it has been excreted. This pheromone(s) is then sensed by the cow by binding to odorant-binding protein (OBP) in the nasal mucosa. The pheromone–OBP complex excites odorant receptor neurons in the main olfactory epithelium or vomeronasal organ, which stimulates mitral tufted cells in either the main olfactory bulb or accessory olfactory bulb. Depending on which bulb is stimulated, the signal is transmitted through the vomeronasal or lateral olfactory tracts, which stimulate the medial or cortical amygdala, respectively. It is within these central nervous system structures that perception occurs by interacting with hypothalamic, hippocampal, and cortical centers that interpret the strength, duration, etc., of the pheromonal signal. Perception of the pheromone stimulates HPO activity by influencing HPA function. Cows perceive pheromones when they are within less than 6 to 8 m of bulls or excretory products of bulls. Perception of pheromones leads to stimulation of pheromonal sensory systems of cows above some yet unknown threshold. As time after calving increases, the threshold for perception and sensory pathways decrease. Each stimulation period is followed by a period of relaxation, and cows respond to this stimulation sooner as the duration of daily stimulation and relaxation cycles increase. The result is an increase in the frequency of LH pulses that is facilitated by activation of the HPA axis. This change in frequency of LH pulses stimulates growth and maturation of a DF, which leads to the preovulatory increase in secretion of estradiol from the DF and the preovulatory release of LH. The LH surge causes ovulation of the DF and formation of a functional corpus luteum, i.e., resumption of ovarian cycling activity.

### FEET ON DRY LAND: THE END OF THE JOURNEY

*Thoughts on the Evolutionary Significance of the Biostimulatory Effect of Bulls.* Why did this arise evolutionarily? I believe that the biostimulatory





**Figure 9.** Conceptual model of hypothetical physiological model by which bulls accelerate resumption of ovarian cycling activity in primiparous, postpartum, anovulatory, suckled cows. HPA, hypothalamic–pituitary–adrenal; HPO, hypothalamus–pituitary–ovarian; MOB, main olfactory bulb; MOE, main olfactory epithelium; MT, mitral tufted; MUP major urinary protein; OBP, odorant-binding protein; ORN, odorant receptor neurons; VNO, vomeronasal organ.

effect of bulls is a “failsafe system” for whenever major internal and environmental effects begin to limit breeding activity in anestrous, postpartum cows. Cows and bulls synchronize the breeding system around a time of the year when survival of the cow and calf is optimum. Cows have a long period of gestation. If postpartum anestrous is extended by major factor limitations, then they will fail to get bred in the breeding season and lose this year to reproduce. Furthermore, if cows are bred late in the breeding season then cows will calve and raise a calf under less than optimal environmental conditions the next year. Cows gain the ability to respond to the biostimulatory effect of bulls after the peak in lactation and once calves begin to fend for themselves. If conditions are limiting, i.e., nutrition or other stress, then these cows become more sensitive to biostimulatory effect of bulls earlier after calving. I think that it is a “redundant system” to ensure that as many females will be bred and calve in the next season as possible. So, what about heifers, why do they show this? But not all do. If major factors are limiting, then heifers do not respond to bull (i.e., decreased growth rate)—the internal hormonal

milieu is insufficient to develop the system that responds to the biostimulatory effect of bulls or genetic controls are not switched on to allow for the development of this system. On the other hand, if internal and external conditions are optimized (i.e., growth rate and the promise of continued nutrients in the environment), then the internal milieu and (or) genetic controls switch on the pheromonal systems necessary for heifers to respond to bulls. Thus, heifers and cows can show such disparate (variation) responses to the biostimulatory effect of bulls. Nevertheless, in my opinion, the biostimulatory effect of bulls ensures sustainable and efficient reproductive performance of anovulatory females under changing environment conditions, which of course leads to optimum survival of the species.

To end, I would like to say that this was a wonderful journey, this career of mine, that had at its foundation love of family and science, and a dedication and commitment to always “endeavor to persevere.” Remember this is a stream of research, as such, this never ends!

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