IMPLICATIONS OF OVARIAN AND UTERINE MATURITY EVALUATED IN REPLACEMENT HEIFER CANDIDATES

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

IMPLICATIONS OF OVARIAN AND UTERINE MATURITY EVALUATED IN REPLACEMENT HEIFER CANDIDATES

presented by Emily Grace Smith

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and hereby certify that, in their opinion, it is worthy of acceptance.

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Dedication

All the work encompassed by this thesis is dedicated to the Lord, in both gratitude for what He has already done through it and submission for what He will do with it.

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List of Abbreviations

ADG	Average daily gain
AI	Artificial insemination
AP	Age at puberty attainment
AFC	Antral follicle count
ARC	Arcuate
AVPV	Anteroventral periventricular
BCS	Body condition score
CL	Corpus luteum
DF	Dominant follicle
E_2	Estradiol
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
HH	Hip height
HPG	Hypothalamic-pituitary-gonadal axis
LFD	Largest follicle diameter
LH	Luteinizing hormone
OS	Ovarian score
PA	Pelvic area
PBE	Pre-breeding evaluation
P_4	Progesterone
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PPI	Postpartum interval
RTS	Reproductive tract score
UHD	Uterine horn diameter
US	Uterine score

Abstract

Profitability across the reproductive lifetime of a cow depends on reproductive success in the first breeding season and requires heifers to attain puberty prior to this time. Pre-breeding evaluations may be used to determine pubertal status of heifers via reproductive tract evaluation. Disparities in ovarian and uterine maturity may be detected at the time of evaluation in peripubertal heifers. Two experiments were conducted to characterize the incidence and implications of disparate ovarian and uterine development during the peripubertal period. Reproductive tract development was assessed, and an individual ovarian score (OS) and uterine score (US) were assigned. Pregnancy outcomes were evaluated on the basis of OS, US and reproductive tract score (RTS: first digit = OS; second digit = US) and relationships between reproductive and physiologic maturity were investigated.

In Experiment 1, duplicate pre-breeding evaluations (PBE) were conducted on 469 heifers approximately 40 and 30 days prior to the start of breeding seasons from 2019-2021. Scores for ovarian (1 = no ovarian development, pea sized; 2 = very few follicles < 8 mm; 3 = several follicles 8-10 mm; 4 = large preovulatory follicle > 10 mm; 5 = corpus luteum present) and uterine (1 = infantile, undeveloped, difficult to palpate; 2 = poorly developed; 3 = distended, moderately developed; 4 = coiled, well-developed, toned; 5 = distended, well-developed) development were assigned following assessment via transrectal palpation and ultrasonography. Uterine horn diameter (UHD), antral follicle count (AFC), largest follicle diameter (LFD), weight, body condition score (BCS), age, and pelvic area (PA) were recorded. Heifers were subjected to the 14-day CIDR-PG estrous synchronization protocol. Split-time artificial insemination was

performed and followed by exposure to bulls 14 days later. Pregnancy diagnosis was performed via transrectal ultrasonography 75-90 days after artificial insemination

In Experiment 2, data from 22,173 heifers custom developed by Heartland Cattle Company from 2014-2018 were analyzed retrospectively. Pre-breeding evaluations were conducted 35-45 days prior to breeding and scores for ovarian (2 = infantile, 3 = no significant structures, 4 = large follicle and/or corpus luteum) and uterine (2 = infantile, 3 = mid-sized, distended tract, 4 = well-vascularized, distended or coiled tract) development were assigned following assessment via transrectal palpation. Weight, hip height, and PA were recorded, and average daily gain (ADG) was calculated for the development period. Heifers were subjected to the 14-day MGA-PG protocol and artificial insemination (AI) were performed based on detected estrus. Pregnancy diagnosis was performed using transrectal ultrasonography 45 days after the end of the breeding season.

The incidence of disparate ovarian score (OS) and uterine score (US) was 33.7% (n = 158/469) in Experiment 1 and 16.3% (n = 3,622/22,174) in Experiment 2. Heifers assigned a RTS of less than 3-3 (Experiment 1 = 4.3% (n = 20/469); Experiment 2 = 0.6% (n = 135/22,174)) demonstrated poor reproductive performance as greater proportions failed to become pregnant in Experiment 1 ($P = 0.03$) and conception to first service AI was decreased in Experiment 2 ($P < 0.01$). Reproductive performance did not differ ($P > 0.10$) between heifers assigned disparate or non-disparate scores of greater than RTS = 3-3. In both experiments, heifers achieving greater physiologic maturity as indicated by age, weight, pelvic area (PA), average daily gain (ADG), or hip height (HH) exhibited greater reproductive maturity as measured by OS and US, respectively.

We propose that disparate ovarian and uterine development is the result of rapid and asynchronous growth of the reproductive tract during the peripubertal period. Consequently, independent assessment of ovarian and uterine maturity may increase precision in characterizing the reproductive maturity of heifers, predicting proximity to puberty attainment, and identifying prepubertal heifers that are unlikely to exhibit satisfactory reproductive performance in the first breeding season.

Chapter 1

Review of Literature

Introduction

Profitability in beef cattle production is maximized when animals and management practices are matched to the resources, systems, and markets of a given context (Short, 2001). Across segments of the beef industry, cost efficiency depends on efficiency of reproduction, producing replacement females, and market animal growth (Dickerson, 1970). In cow-calf production, feed is the greatest contributor to cost (USDA-ERS, 2011), and revenue is largely generated by the production of calves. Though replacement females will begin to generate revenue with the production of their first calf, costs incurred during development will generally exceed income, and thereby prevent profit, in the first several years of production. Consequently, profitability of females in a cow-calf production system is closely related to age at first calving and length of productive life (Kress et al., 1969).

Heifers that calve for the first time at two years of age have the potential to produce more calves over their reproductive lifetime than those that calve for the first time at greater ages (Rice et al., 1961; Donaldson et al., 1968; Pinney et al., 1972; Bernard et al., 1973; Carter and Cox, 1973; Cundiff et al., 1974; Morris, 1980; Nunez-Dominquez et al., 1991). In the context of commercial systems managing for a fixed-length breeding season, heifers that calve early in their first calving season remain in production for a greater number of years (Cushman et al., 2013). Achieving adequate pregnancy rates among yearling heifers requires a large proportion of the herd to attain

puberty prior to the first breeding season (Short and Bellows, 1971). The age at which a heifer attains puberty is determined by the influence of environmental inputs on the expression of her innate genetic potential.

Heifer development is one of the greatest control points of profitability in cow-calf production (Meek et al., 1999), but the degree of variation demonstrated in both input cost and animal performance results in a necessity for contextualization of development practices. Alignment of heifer selection and management to the context of development must be founded on an understanding of the physiological processes required for puberty to be attained.

Physiology of Puberty Attainment

In heifers, a number of reproductive structures and processes are already functional or may be induced at birth. Competence of reproductive systems has been demonstrated in neonatal animals via administration of exogenous hormones or removal of reproductive and endocrine tissues. Ovulation of fertile ova may be stimulated as early as one month of age via injection of gonadotropins (Seidel et al, 1971). Administration of supraphysiological levels of estradiol (E_2) can induce surges of luteinizing hormone (LH) mimicking those that precede ovulation in three- to five-month-old heifers (Staigmiller et al., 1979; Schillo et al., 1982). However, in order for these events to occur spontaneously and culminate in puberty attainment, a complex maturation process must occur both anatomically and endocrinologically.

Endocrinologic Regulation of Puberty

Endocrine regulation of puberty depends on dynamic interactions between gonadotropins produced in the hypothalamic pituitary axis and ovarian-derived steroids. Rapid changes in the frequency, amplitude, and concentration of these hormones between birth and puberty establish and alter feedback pathways that will regulate the estrous cycle in mature animals. Pulsatile release of LH from the pituitary of beef heifers increases in amplitude from birth to one month of age, before declining to a sustained level (Schams et al., 1981). Despite decreasing amplitude, circulating concentrations rise as pulse frequency increases to a peak at three months of age, before declining until 6 months of age (Schams et al., 1981). Approximately 50 days prior to puberty, pulse frequency rises until a rate of one pulse per hour is achieved preceding the events of an initial ovulation (Kinder et al., 1987).

In the prepubertal period, pulsatile release of LH occurs in response to the pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus (Skaggs et al., 1986). A threshold concentration of GnRH is necessary to induce LH pulsation, but direct correlations in concentrations above threshold are not observed. Gonadotropin-releasing hormone is also responsible for triggering release of follicle-stimulating hormone (FSH), which is believed to play a passive role in the process of puberty attainment (Gonzalez-Padilla et al., 1975a). Following an initial rise to peak circulating concentration at one month of age, FSH levels decline at two months of age and stabilize until the first ovulation (Desjardins and Hafs, 1968). Both GnRH and FSH remain consistent throughout the prepubertal period for any individual, but marked variation is observed between heifers (Schams et al, 1981; Gonzalez-Padilla et al., 1975).

Immediately preceding puberty, slight declines in GnRH and FSH are correlated to a contemporaneous rise in circulating progesterone (P_4) concentration; even so, it is unlikely that this interaction is significant to puberty attainment (Gonzalez-Padilla et al., 1975a; Skaggs et al., 1986).

The classic "gonadostat" theory was initially proposed by Ramirez and McCann (1968) as the physiological mechanism of puberty attainment following studies in mice. Observation of LH concentrations following administration of estradiol (E_2) to ovariectomized mice revealed E_2 as inhibitory to LH synthesis and secretion in prepubertal animals. Though E_2-mediated inhibition was observed in all mice in the study, feedback sensitivity was two to three times greater in immature than mature mice. However, the inhibitory influence of E_2 on LH release diminishes approaching puberty, despite increases in circulating E_2 concentrations. These changes were demonstrated in ewe lambs by Foster and Ryan (1979), as sensitivity to E_2 administration was observed to decrease approaching puberty attainment. Schillo et al. (1982) correlated heifer maturity with sensitivity to E_2 feedback by demonstrating that younger calves exhibit longer periods of LH suppression following E_2 administration.

A decline in the capacity of hypothalamic neurons to bind to E_2 at the time of puberty attainment provided an initial explanation for this phenomenon (Day et al., 1987). However, GnRH neurons lack the estrogen receptor necessary (ERα) for direct stimulation of GnRH release to be the principal mechanism (Skynner et al., 1991). Ongoing research surrounding relationships between E_2 and the neurons located in the arcuate nucleus (ARC) and preoptic area (POA) of the hypothalamus continues to uncover complex mechanisms for mediation of feedback between E_2 and GnRH.

The neuropeptide kisspeptin was first implicated as a mediator of puberty attainment following observation of an increasing response of GnRH neurons to kisspeptin stimulation in mice approaching puberty (Han et al., 2005). Subsequent observation of neurokinin B (NKB) and dynorphin (DYN) expression from kisspeptin producing neurons in the ARC nucleus (Goodman et al., 2007) facilitated recognition of KNDy neurons (Lehman et al., 2010). These neurons express ERα (Franceschini et al., 2006), generate GnRH pulsatility in mature animals (Goodman et al., 2013), and increase kisspeptin production in the ARC nuclei and POA following a surge of E_2 (Smith et al., 2009), suggesting an active role in induction of the LH surge (Smith et al., 2009) and puberty attainment (Lehman et al., 2010). However, recent studies in ewe lambs contradict a temporal correlation to puberty attainment as production of all three proteins is fully functional in prepubertal animals (Aerts et al., 2021) and notable changes are not observed in number, production, or ERα expression of KNDy neurons in the ARC nucleus approaching puberty (Bedenbaugh et al., 2018, Aerts et al., 2021). The principal drivers of neuronal maturation processes that precede puberty attainment have yet to be verified in ruminants. However, ongoing areas of research center around upstream regulators of kisspeptin production including proopiomelanocortin (PPO), agouti-related peptide (AgRP), neuropeptide Y (NPY), and α-melanocyte stimulating hormone (α-MSH) as potential candidates (Cardoso et al., 2018, Aerts et al., 2021).

One of the most significant consequences of puberty attainment is the marked rise in P_4 that results from formation of a corpus luteum (CL) on the ovary following the first ovulation. Prior to the existence of this transient endocrine structure, P_4 is primarily of adrenal origin and circulating concentrations remain low with little to no variation early

in life (Wagner et al., 1972). In the 20 days preceding puberty, circulating progesterone concentrations exhibit two transient elevations of increasing magnitude (Gonzalez-Padilla et al., 1975a). Debate surrounds both the source and function of these P_4 elevations. However, observation of the direct relationship between preovulatory P_4 concentrations, the LH-binding capacity of follicular cells, and the amount of E_2 secreted from pre-ovulatory follicles (Hunter and Southee, 1987) has precipitated the hypothesis that P_4 exposure is necessary to prime the ovary to respond to the LH surge (Gonzalez-Padilla et al., 1975a). The sequence of LH and P_4 peaks suggests that the first rise in P_4 may be derived from microscopically detectable luteal tissue in ovaries lacking ovulatory papillae or corpora lutea (Berardinelli et al., 1979; Kinder et al., 1987). The second rise is often attributed to the formation of a luteal structure following an ovulation that occurs without behavioral estrus expression. (Berardinelli et al., 1979, Moran et al., 1989). These "silent" ovulatory events result in short-lived corpora lutea and abnormal, short cycles preceding the first complete estrous cycle. Conversely, estrus expression may occur in the absence of ovulation. This phenomenon, termed nonpuberal estrus, is more commonly associated with lighter or younger heifers (Nelson et al., 1985; Rutter and Randel, 1986) and is often associated with low circulatory P_4 preceding ovulation (Rutter and Randel, 1979). Given the hypothesized priming effect of P_4, these low concentrations may be responsible for ovulation failure in heifers expressing nonpuberal estrus.

Reproductive Tract Development

Reproductive success depends not only on successful puberty attainment, but on appropriate maturation of the reproductive tract. Development of reproductive tissues is essential to establishing pregnancy, accommodating fetal growth, and successfully

completing the first parturition. The gross anatomy of the ovaries, oviduct, uterus, cervix, and vagina is established during prenatal development, but growth of these tissues occurs at variable rates relative to that of body weight during the postnatal period (Desjardin and Haf, 1969).

Immediately following birth, ovarian weight increases at a rate four times that of body weight, as a consequence of the proliferation of vesicular follicles within the ovaries (Desjardin and Haf, 1969; Honaramooz et al., 2004). Immediately preceding puberty, a second period of ovarian growth is likely the effect of interstitial growth and increasing follicular size, as the number of vesicular follicles does not increase (Desjardin and Haf, 1969). The biphasic growth pattern of the tubular reproductive tract exhibits strong correlation to increasing follicular diameter, suggesting a responsiveness to E_2 produced by developing follicles (Honaramooz et al, 2004). Hypertrophic and hyperplastic processes allow uterine weight to increase at a greater rate than body weight (Desjardin and Hafs, 1969). Vaginal and oviductal weight increase in proportion to body weight, while increases in cervical weight occur at a more rapid rate (Desjardin and Hafs, 1969).

Neonatal bovine uteri contain a complete number of caruncles; these structures flatten to reflect their mature conformation prior to puberty attainment (Atkinson et al., 1984). Maturation of the uterine glands in the intercaruncular region of the endometrium is independent of tissue growth (Atkinson et al., 1984). Development is initiated in heifer calves during the final month of gestation, potentially in response to elevated levels of maternal E_2 (Atkinson et al, 1984), and continues throughout neonatal uterine growth. Well-developed uterine glands are present at 5-6 months of age (Desjardin and Haf, 1969).

Follicular Development

Oogenesis is initiated early in gestational development with the migration of primordial germ cells to the gonadal ridge (Erickson, 1966). Clusters of primordial germ cells disperse throughout the ovarian cortex and associate with somatic cells to form germ cell cysts (Erickson, 1966). Primordial germ cells undergo mitotic proliferation before entering the first meiotic division that marks oocyte differentiation. An individual oocyte surrounded by a layer of squamous somatic cells constitutes a primordial follicle (Rajakoski, 1960). Oocytes progress to the pachytene stage of prophase of the first meiotic division where they remain arrested in development until factors produced during the estrous cycle allow meiosis to resume following puberty attainment (Henricson and Rajakoski, 1959).

Continuous turnover of primordial follicles begins prior to birth as subsets of follicles progress through stages of growth and atresia (Erickson, 1966). Follicular growth is marked by differentiation of three distinct somatic cell populations – granulosa, theca interna, and theca externa cells (Rajakoski, 1960). Somatic cells respond to local influences and stimuli from the HPG, produce regulatory factors responsible for control of the estrous cycle, and provide various supportive functions for developing oocytes (McNatty et al., 1984). Evolution of the granulosa cell layer from squamous to cuboidal structure signifies the transition between primordial and primary follicles. Secondary follicles are identified by two to three layers of granulosa cells and the presence of a single layer of theca interna cells separated from granulosa cells by a thin membrane known as the lamina propria. The defining characteristic of the tertiary stage is

identification of an antrum created by the accumulation of follicular fluid. Theca externa cells may also be observed surrounding the theca interna cells at this stage.

Primordial follicle numbers are greatly reduced by follicular turnover throughout prenatal development. The population of primordial follicles remaining at birth, known as the ovarian reserve, supplies oocytes for individual estrous cycles throughout the reproductive life of the cow. The number of follicles growing simultaneously on the ovary at any given time, measured as the antral follicle count (AFC), is directly correlated to the ovarian reserve (Ireland et al., 2008). Antral follicle count rises rapidly during neonatal development but stabilizes prior to puberty and remains consistent well into productive years (Katska et al., 1984). Though AFC is consistent over time for an individual animal, both ovarian reserve and AFC vary dramatically between individuals (Rajakoski, 1960; Erickson, 1966).

Follicular waves closely resembling those of mature animals are established in concert with increasing gonadotropin concentrations as early as two weeks of age (Evans et al., 1994; Honaramooz et al., 2004). Follicular waves continue to turn over in response to low levels of FSH and LH prior to puberty. Despite the absence of the suppressive effects of P_4, low LH pulse frequencies in prepubertal heifers result in waves of shorter duration than those of mature animals (Adams et al, 1994).

Follicular waves are classically divided into three phases: recruitment, selection, and dominance (Hodgen, 1982). The expression of FSH receptors on granulosa cells (Bao et al., 1997) allows folliculogenesis to begin in a small cohort of follicles following a rise of FSH (Fortune et al., 2000). The necessity of FSH for this recruitment event marks the transition from gonadotropin-independent to gonadotropin-dependent growth (Lucy et al.,

1992). Follicle-stimulating hormone upregulates the synthesis of steroidogenic enzymes, such as the cholesterol-side chain cleavage enzyme and aromatase, resulting in production of low levels of E_2 which can be noted in systemic circulation and follicular fluid (Bao and Garverick 1997;1998). Inhibin produced by granulosa cells exerts an inhibitory effect on the release of FSH from the HPG (Kaneko et al., 1995). Concentrations of E_2 and inhibin parallel follicular growth (Mihm, 2000). The expression of LH receptors increases slightly following recruitment but remains confined to theca cells (Bao and Garverick, 1997). Low systemic LH concentrations, a scarcity of LH receptors, and observation of steadily increasing FSH pulses during this period suggests FSH as the predominant stimulant for recruitment (Bao and Garverick, 1997). However, falling FSH concentrations due to growing follicular E_2 and inhibin production results in lack of stimulus for continued development in the absence of alternative stimulation.

Selection is the process by which a single follicle becomes dependent on LH through one of several proposed mechanisms, allowing a dominant follicle (DF) to deviate from the developing cohort. The Missouri model of selection proposed that the DF escapes atresia via acquisition of LH receptors on granulosa cells (Bao and Garverick, 1997). In this model, dramatic increases in E_2 and inhibin production by the first follicle to acquire LH receptors go on to exacerbate FSH inhibition, starving all other follicles in the cohort. Alternatively, the Cornell model asserted that a single follicle possessed a developmental advantage accompanied by greater availability of insulin-like growth factor I from the onset of recruitment. In this model, insulin-like growth factor I stimulates E_2 and inhibin production, providing the negative feedback necessary to starve other cohort follicles (Evans and Fortune, 1996). Contemporaneous increases observed in

LH receptor expression and insulin-like growth factor I suggest that the mechanisms proposed by both models are synergistic in the selection process (Beg and Ginther, 2006).

The DF has several unique and crucial roles in modulating endocrine patterns during the estrous cycle and supporting oocyte maturation (Pincus and Enzmann, 1935). Functional dominance is exhibited in suppression of subordinate follicles via the negative feedback of E_2 and inhibin. Though functional dominance terminates when steroidogenesis ceases (Fortune et al., 1991), morphologic dominance, defined as the period during which the DF can be identified as the largest follicle, may extend beyond this point. In a low P_4 environment, the DF will progress to a preovulatory stage characterized by rampant production of E_2 (Bao and Garverick, 1998). In prepubertal heifers, this increase in E_2 is essential for maturation of the HPG (Dodson et al., 1989). A high P_4 environment will suppress LH pulse frequency, permitting the DF to undergo atresia (Sirois and Fortune, 1990).

Atresia is an irreversible process of regression that may occur in follicles at any stage of growth (Marion et al., 1968). It is observed in preantral follicles that lack FSH stimulation for antral growth at the time of recruitment and in subordinate follicles that lack LH stimulation at the time of selection. Inadequate receptor expression, deficient gonadotropin concentration, and inhibition from ovarian derived factors may all contribute to initiation of atresia (Bao and Garverick, 1998).

Regression begins with apoptosis of granulosa cells following concurrent loss of antiapoptotic signals and binding of proapoptotic ligands (Matsuda et al., 2012). Endogenous apoptotic pathways are constitutively active in the absence of antiapoptotic factors and binding of death ligands hastens regressive processes. Though intermediate

steps vary between pathways triggered by loss of antiapoptotic factors and gain of proapoptotic factors, both result in activation of caspases and an irreversible trajectory towards cell death (Matsuda et al., 2012).

Morphologically, atresia occurs in basal and antral forms (Irving-Rodgers et al., 2001). Preantral and early antral follicles typically regress through basal atresia while antral atresia is the primary form for larger follicle classes (Irving-Rodgers et al., 2001). Basal atresia is characterized by apoptosis of the granulosa cells closest to the lamina propria and theca cells. As death of basal granulosa cells progresses, degeneration of the lamina propria and breaching of proximal capillaries, allows invasion of macrophages for phagocytosis of dead cells and debris (Irving-Rodgers et al., 2001). Antral atresia is initiated in the cells nearest the antrum and is characterized by accumulation of sloughed granulosa cells in the follicular lumen. Degeneration of the lamina propria and thecal cells occurs when a single layer of granulosa cells remains (Marion et al., 1968). Atresia of large, well-vascularized follicles is accompanied by sclerotization and hyalinization of proximal capillaries (Marion et al., 1968). Production of steroidogenic enzymes is drastically reduced prior to onset of morphological atresia, and steroidogenic capacity decreases throughout regression (Bao and Garverick, 1998). Estradiol concentrations fall with the loss of healthy granulosa cells, and androgen concentrations in follicular fluid wane with thecal cell degeneration (Irving-Rodgers et al., 2001).

The First Ovulation

If LH stimulus is sufficient, folliculogenesis may culminate in ovulation of the DF and release of a mature oocyte. A cascade of hormonal changes occurs in the several days preceding this event. A lack of the inhibitory effects of P_4 in prepubertal heifers permits

follicular growth (Adams 1992) and the resultant increasing in E_2 production (Evans et al., 1994). Positive feedback of E_2 at the HPG induces the LH surge, which serves as the endocrine stimulus most directly responsible for ovulation (Henricks et al., 1970, Chenault and Thatcher, 1975). Luteinizing hormone receptor concentrations in granulosa and theca cells increase at this time (Webb et al., 2003), priming both cell populations to respond by activating cyclic adenosine monophosphate mediated pathways. These pathways directly inhibit production of androgen substrates in the E_2 synthesis pathway (Dieleman et al., 1983) and stimulate production of enzymes necessary for P_4 production. Therefore, E_2 synthesis declines while P_4 production increases (Murdoch, 1985), facilitating production of both prostaglandin F_{2a} ($PGF_{2\alpha}$) and prostaglandin E (Wise et al., 1986; Fortune et al., 2009). These forms vary from one another in action, yet both prostaglandins induce functions that conflict by simultaneously stimulating and inhibiting ovulation. Questions remain regarding the role of prostaglandins in ovulation in cattle (Berisha et al., 2019). Although beyond the scope of this review, cellular mechanisms regulating steroidogenesis and ovulation have been reviewed in detail by Lipner (1973).

These endocrine events regulate physical changes necessary for follicular rupture. Estradiol increases blood flow to the follicular wall (Acosta et al., 2003), and the LH surge triggers vasodilation of the microvasculature of the connective tissue in the theca interna. The resulting vascular damage, extravasation of blood fractions and immune cells, and hyperemia allow an accumulation of follicular fluid that exerts a static pressure on the follicular wall (Cavender and Murdoch, 1988). Prostaglandins stimulate the production of proteolytic enzymes, such as collagenase, which degrade the connective tissue of thecal layers and weaken the follicular wall (Rondell, 1974, Murdoch, 1985).

Pressure-induced local ischemia and enzymatic degradation permit a breach in the wall of the follicle at the stigma, which may be identified prior to ovulation (Rondell, 1970; Cavender and Murdoch, 1988).

The Corpus Luteum

The preovulatory LH surge begins the process of follicular cell luteinization (Donaldson and Hansel, 1968). Immediately following ovulation, collapse of the follicular wall results in invagination of the theca interna, along with its associated vasculature and connective tissue, into granulosa cell layers (Loeb, 1906). Degeneration of the lamina propria results in the formation of a diffuse population of highly mitotic theca and granulosa cells, permitting a heterogenous population of steroidogenically active cells to characterize the mature CL (Donaldson and Hansel, 1965). Follicular cells undergo hypertrophy before differentiating into luteal cell populations (Priedkalns and Weber, 1968). Small luteal cells are generated from theca interna cells, while large luteal cells may originate directly from granulosa cells or indirectly from theca cells as small luteal cells may evolve into large luteal cells (Alila and Hansel, 1984).

Luteal cells respond to LH stimulus in a similar fashion to their follicular cell predecessors by activating cyclic adenosine monophosphate mediated pathways (Marsh and Savard, 1966) for enzyme synthesis and acquisition of P_4 precursors (Niswender et al., 1981). Steroidogenic capacity is determined by both LH release (Donaldson and Hansel, 1965) and the degree of receptor binding (Richards et al., 1976). Basal levels of LH are required to maintain CL function in large and small luteal cells (Peters et al., 1994), but small luteal cells also demonstrate a dose-dependent response in P_4 production (Donaldson and Hansel, 1965; Ursely and Leymarie, 1979). The rate of P_4 production is

limited by the availability of oxygen and substrates for steroid production and therefore dependent on proximity of steroidogenic cells to luteal capillaries (Janson et al., 1981; Farin et al., 1986). Release of angiogenic factors from inflammatory and luteal cells in the periovulatory period facilitates the formation of an extensive microvascular network from endothelial cells housed in theca-derived connective tissue (Dondaldson and Hansel, 1965; Gospodarowicz and Thakral; 1978, Amselgruber et al., 1999). Luteal cells continue to direct the formation and maintenance of capillaries in response to the metabolic demands of steroidogenesis over the lifespan of the CL (Gospodarowicz and Thakral, 1978). Steroidogenic capacity is directly correlated to the size and perfusion of the pre-ovulatory follicle (Tarso et al., 2018) and the size of the CL during the growth phase (Mann, 2009). However, P_4 release by the mature CL is more related to blood flow than CL size (Herzog et al., 2010).

Prostaglandin $F_{2\alpha}$ is the factor responsible for induction of luteolysis in the cow (Rowson et al., 1972). This prostaglandin is produced by the uterus and reaches the ovary through local vascular pathways (Anderson et al., 1962). Close association between the uterine venous drainage system and the ovarian artery allows lipid-soluble $PGF_{2\alpha}$ leaving the uterus to diffuse into ovarian circulation for transport to the CL (Hixon and Hansel, 1974). The vasoconstrictive effects of $PGF_{2\alpha}$ reduce ovarian blood flow and induce functional regression, resulting in a decline in P_4 production (Nett et al., 1976). Morphological changes associated with functional regression include sclerotization of ovarian arteries and peripheral vacuolation of luteal cells. Structural regression is characterized by nuclear degeneration, cytoplasmic condensation, and phagocytic removal of luteal cell fragments (Donaldson and Hansel, 1965; Juengel et al., 1993).

Factors that Influence Puberty Attainment

When the first ovulation, expression of estrus, and normal luteal phase occur in appropriate succession, puberty is attained (Moran et al., 1989). The age at which a heifer attains puberty is determined by the influence of environmental factors and management decisions on the expression of her innate genetic potential.

Genetics

Genetic variation in age at puberty attainment (AP) may be observed both within and between breeds. Though heritability of reproductive traits is generally low, AP is moderately heritable (0.40) and may be selected for with reasonable accuracy (Martin et al., 1992; Cammack et al., 2009). Genetic correlations between traits results in concomitant shifts in reproduction, growth, and carcass merit with selection for any individual attribute (Martin et al., 1992). Selection for a variety of these traits over many generations has resulted in genetic similarity among certain breeds that may be grouped into biological types.

Biological types differ in AP (Martin et al., 1992). Breeds with smaller mature sizes tend to attain puberty at younger ages, and those selected for milk production tend to demonstrate further reductions in AP independent of size (Gregory et al., 1979). Additionally, individual genetic variation contributes to the rate of reproductive maturation as demonstrated by variation in AP of progeny between individual sires of a given breed (Laster el., 1976). At the level of both biological type and individual sires, AP is likely to increase with selection for weight or growth (Brinks et al., 1964; MacNeil et al., 1984; Wolfe, 1990) and decrease with selection for milk production (Gregory et al., 1979). Mature size is inversely related to rate of physiologic maturation (Nelsen et al.,

1982). However, among heifers of a similar expected mature size, those that exhibit greater growth rate relative to mature size will attain puberty at younger ages (Smith et al., 1976).

Crossbreeding has remarkable effects on AP, via both breed complementarity and heterosis. Crossbred heifers benefit from additive genetic effects by achieving puberty at an intermediate age to that expected of the sire and dam breeds. Nonadditive effects are realized in the form of heterosis and maternal effects. Crossbred heifers are younger than straightbred contemporaries in both direct comparison (Wiltbank et al., 1966; Arije and Wiltbank, 1971; Laster et al., 1976; Gregory et al., 1978) and after adjusting for heterotic influence on heifer weight and rate of gain (Wiltbank et al., 1966; Gregory et al., 1978). Maternal effects on heifer AP are a combination of the dam's genetic merit and the environmental influence attributable to the dam (Tyler et al., 1947; Gregory et al., 1978), primarily in the form of milk production (Notter et al., 1978). Distinguishing between the mechanisms of maternal effects is challenging (Willham et al., 1980); however, regardless of the source of influence, progeny from dam breeds with greater levels of milk production are likely to attain puberty earlier (Gregory et al., 1978). Though genetic composition is the largest contributor to an individual's minimum AP, differences observed among genetically similar animals reaffirms that genetic variation is not the sole determinant of puberty attainment.

Nutrition

Nutritional influence on puberty attainment is likely mediated by neuroendocrine communication at the HPG (Cardoso et al., 2018). Leptin, a protein hormone released from adipose tissue, is a proposed intermediary between nutrition and reproduction in

cattle (Zieba et al., 2005). Nutritional signals are directly conferred to the HPG where neuropeptide Y, proopiomelanocortin, and kisspeptin neurons in the ARC nucleus express leptin receptors (Allen et al, 2012). In heifers, circulating leptin concentrations are correlated to both transient nutrient availability (Amstalden et al., 2000) and body energy reserves (Chilliard et al., 2005). Nutrient restriction in the peripubertal period can decrease responsiveness to GnRH and delay the increase in LH pulsatility that marks the onset of puberty attainment (Day et al., 1986). A high plane of nutrition results in greater circulating leptin concentrations during preweaning and postweaning phases of development (Cardoso et al., 2014; Bruinjé et al., 2021). Increases in leptin stimulate downstream release of GnRH (Day et al., 1986), which may reduce the negative feedback of E_2 and permit the increase in LH necessary for the first ovulation (Gasser et al., 2006a).

It is plausible that a heifer's degree of adiposity is a regulator of puberty attainment (Ferrell, 1982; Brooks et al., 1985; Nelsen et al., 1982; Perry, 2016). This hypothesis is consistent with the greater variability in AP observed among undernourished animals compared to those for which nutrient requirements have been met (Wiltbank et al., 1966, 1969). Heifers consuming restricted diets, exhibiting low rates of gain, or with minimal stored fat exhibit greater averages and wider ranges in AP (Short and Bellows, 1971).

Differences in the effects of nutrient restriction by age suggest that adaptations in neuronal networks and feedback pathways depend on a heifer's physiologic maturity at the time of restriction. Among non-restricted heifers, the timing with which weight is gained does not influence AP (Lynch et al., 1997, Cardoso et al., 2014). However, when

weight gains are insufficient for fat accretion, timing of gains may influence AP (Wiltbank et al., 1966; Dofour, 1975; Cardoso et al., 2014). In these instances, preweaning nutrition may exert greater influence on AP than postweaning nutrition (Arije and Wiltbank, 1971; Laster et al., 1976; Cardoso et al., 2014). It has been hypothesized that hypothalamic neuronal pathways undergo restructuring as a result of nutritional programming during the preweaning period (Cardoso et al., 2014). Feeding heifers high energy diets at this time may reduce average age at puberty (Cardoso et al., 2014) and has even been shown to induce precocious puberty (Gasser et al., 2006b). In the absence of nutritional restrictions on weight gain, animals fed at higher planes of nutrition often attain puberty at greater weights than those fed at lesser planes of nutrition (Wiltbank et al., 1966; Short and Bellows, 1971; Nelsen et al., 1982; Schillo et al., 1983; Moseley et al., 1982; Hall et al., 1995; Martin et al., 2008). This observation supports the notion that body weight and rate of gain are not the primary determinants of age at puberty attainment if energy reserves are adequate (Wiltbank et al., 1966, 1969; Short and Bellows, 1971; Moseley et al., 1982; Hall et al., 1995).

Studies examining relationships between reproductive performance and postweaning nutrition have precipitated varied recommendations for the degree of physical maturity that should be achieved by the first breeding season. Early studies indicated that development of heifers to less than 60-65% of their expected mature weight might result in reduced proportions of the herd attaining puberty, lesser pregnancy rates, increased incidence of dystocia, greater postpartum interval, and reduced calf weaning weights (Bellows et al., 1982; Wiltbank et al., 1985; Patterson et al., 1989, 1991). More recently, studies have indicated that heifers may be developed to 50-55% of

their mature body weight without significantly impairing reproductive performance (Funston et al., 2012). Despite delays in puberty attainment and conception during the breeding season, heifers developed to a lesser percentage of mature weight demonstrated similar first service conception rates and final pregnancy rates to those developed to greater levels of maturity (Funston and Deutscher, 2004; Salverson et al., 2005; Martin et al., 2008; Roberts et al., 2009; Funston and Larson, 2011). Conclusions from these studies may be closely tied to the biological type of heifers and system context studied; additional research is warranted to evaluate the effects of development to a lesser percentage of mature body weight in other contexts and with other reproductive strategies (Funston, 2012).

The composition of nutrients in a diet may have an effect on puberty attainment independent of dietary energy by influencing maturation of the HPG via acute or sustained changes in metabolic signaling (Marston et al., 1995). Modification to the starch content of the diet influence the ratio of propionate to acetate in the rumen (Krause et al., 2003). Increases in this ratio have been shown to enhance sensitivity of the anterior pituitary to GnRH (Randel et al., 1980), increase ovarian follicular response to gonadotropin stimulation (Bushmich et al., 1980), and hasten puberty attainment (McCartor et al., 1979; Gasser et al., 2006b). Thus, heifers fed high-starch diets may attain puberty earlier than those fed low-starch diets, even when energy intake and body weight are unaffected (Ciccioli et al., 2005).

Feed additives may influence puberty attainment via the same mechanism. Monensin is an ionophore fed to increase feed efficiency by altering the ruminal propionate to acetate ratio (McCartor et al., 1979). Herds fed monensin demonstrated an

increase in the proportion of heifers attaining puberty prior to breeding (Moseley et al., 1977) and a decrease in average AP (McCartor et al., 1979). Though this influence was independent of nutrition level (Moseley et al., 1982), effects of may be magnified in nutrient-restricted heifers as the growth promoting action of monensin may permit weight gains (Moseley et al., 1977, 1982; Bushmich, 1980).

As puberty attainment is predicated on achieving adequate weight, dietary protein must be provided at a level sufficient to meet requirements for growth. However, excessive supplementation of protein has been shown to impair follicular growth and retard puberty attainment (Lalman et al., 1993; Kane et al., 2004). Similarly, fat supplementation may confer reproductive advantages when it serves as an energy source to animals otherwise at a deficit (Funston et al., 2004), but not when provided at levels beyond energy requirements (Lammoglia et al., 2000; Whitney et al., 2000). Some studies indicate that specific fatty acids provided in ruminally protected feed sources may improve conception rates and reduce early embryonic loss in postpartum cows (Cooke, 2019). These effects have yet to be evaluated in heifers, and relationships between specific fatty acids and puberty attainment have not been observed (Shike et al., 2013; Hall et al., 2015).

Environment

Though cattle are not seasonal breeders, considerable evidence suggests that season of birth influences the timing of puberty attainment. The physiologic mechanisms responsible for seasonal effects have yet to be elucidated (Hansen, 1985), but studies have demonstrated the influence of photoperiod on puberty attainment (Hansen et al., 1983). Observation of increased LH in response to E_2 administration among heifers

exposed to increased periods of light suggests that photoperiod may accelerate the escape of the HPG from the negative feedback of E_2 (Hansen et al., 1982). The magnitude of these effects varies between biological types, with significantly greater photoperiodic dependence observed in *Bos indicus* than *Bos taurus* heifers (Rhodes et al., 1982; Randel, 1984). For all biological types, it is reasonable to conclude that seasonal influences predispose cattle to calve in the spring (Schillo et al., 1983; Randel, 1984; Hansen, 1985). Though puberty attainment may be delayed by winter (Grass et al., 1982) and accelerated by summer conditions (Hansen et al., 1982, Schillo et al., 1983), definitive correlations between season and AP remain elusive, as contradictory studies have identified earlier puberty attainment for heifers born in both spring (Menge et al., 1960; Roy et al., 1980) and fall (Schillo et al., 1982).

Biostimulation has been defined as the "stimulatory effect of a male on estrus and ovulation in the female via pheromones, genital stimulation, or other less well-defined external cues" (Chenoweth, 1983). In cattle, biostimulatory effects are largely attributed to the perception of pheromones by the olfactory system (Johns, 1980; Rekwot et al., 2001). Circulating LH concentrations are altered shortly after bull exposure (Fiol and Ungerfeld, 2016), but variation in reproductive performance is not realized until much later time points (Fiol and Ungerfeld 2010, 2016; Ungerfeld, 2018). These delays in reproductive effects support the hypothesis that the physiological mechanism of biostimulation centers on accelerating maturation of the HPG.

The effectiveness of biostimulation for hastening puberty attainment may vary based on heifer nutrition as well as the form, duration, and intensity of the male stimulus. In some studies, greater proportions of heifers attained puberty when herds were exposed

to bull urine (Izard and Vandenbergh, 1982), mature bulls (Roberson et al., 1991; Oliveira et al., 2009), teaser bulls (Pfeiffer et al., 2011), or androgen-treated steers (Fiol et al., 2010; Fiol and Ungerfeld, 2016). Other studies demonstrated no difference in puberty attainment among heifers exposed to teaser bulls (Berardinelli et al., 1977), vasectomized bulls (Pfeiffer et al., 2011), or mature bulls (Roberson et al., 1987). Failure of bull exposure to hasten puberty attainment among marginally developed heifers in some studies suggests a possible interaction between biostimulation and rate of growth (Roberson et al., 1991; Oliveira et al., 2009; Pfeiffer et al., 2011; Fiol et al., 2010).

Challenges in Heifer Development

Breeding yearling heifers

Pregnancy rates depend on the proportion of the herd attaining puberty prior to breeding (Short and Bellows, 1971) and greater reproductive performance has been observed among heifers bred after their puberal estrous cycle (Byerley et al., 1987). As pregnancy outcomes are highly correlated to animal maturity, these observations may be attributable to increases in heifer age and weight that correspond with the number of estrous cycles experienced (Roberts et al., 2019). Though heifer age cannot be manipulated, puberty attainment and reproductive outcomes may be impacted by management strategies designed to accelerate growth.

Commercially available implants containing combinations of natural and synthetic estrogenic and anabolic compounds are implemented in the beef industry to improve growth and feed conversion efficiency (Duckett et al., 1996). However, the use of growth-promoting implants in replacement heifer candidates is questioned, as studies have demonstrated negative impacts on reproductive performance. As the steroidogenic

compounds in implants are involved in natural maturation processes, impacts may occur prior to puberty via altered thresholds in the feedback pathways of E_2 and LH (Kniffen et al., 1999) or following puberty via impaired uterine gland development and reduced functional capacity (Bartol et al., 1995).

In some instances, growth implants were associated with impaired reproductive tract development (Moran et al., 1990), reduced proportions of heifers attaining puberty prior to breeding (Heitzman et al., 1979; Duetscher et al., 1986; Kniffen et al., 1999; Moran et al., 1990; Devine et al., 2015), increased incidence of non-puberal estrus (Deutscher et al., 1986; Moran et al., 1990), reduced pregnancy rates (Selk, 1997), and reduced endometrial gland density (Bartol et al., 1995). Other research concluded that implanted heifers did not differ in puberty attainment (Staigmiller et al., 1986) or pregnancy outcomes (Heitzman et al., 1979; Staigmiller et al., 1983; Deutscher et al., 1986; Devine et al., 2015) compared to non-implanted heifers.

The effect of implants on reproductive processes depends on the time at which heifers are implanted and the number and type of implants received prior to puberty (Deutscher et al., 1986; Moran et al., 1990). The growth benefits of implants are maximized when products are administered prior to weaning and reimplanted in series (Jones, 2014). However, the negative impacts on reproductive performance were greatest among animals implanted prior to weaning (Deutscher et al., 1986; Cohen et al., 1987; Selk, 1997), as this developmental period is characterized by the greatest degree of plasticity and steroid sensitivity (Bartol et al., 1995).

Complete avoidance of implants in heifers may be economically inefficient and overly cautious in contexts in which the revenue generated from increased gains exceeds

that lost by reduced conception rates (Gutierrez et al., 1995; Clark et al., 2005). As potential risk to reproductive performance decreases dramatically after 2-3 months of age (Deutscher et al., 1986; Cohen et al., 1987; Selk, 1997), implants may be judiciously used in systems in which the number of heifers developed exceeds the desired replacement rate (Gutierrez et al., 1995).

Direct induction of puberty attainment is possible via administration of exogenous progestins (Short et al., 1976; Gonzalez-Padilla et al., 1975b; Burfening, 1979; Sheffield and Ellicott, 1982). Progestins may be effectively administered to heifers via insertion of an intravaginal controlled internal drug release (CIDR) device containing P_4 (Lucy et al., 2001) or consumption of the orally active P_4 analog melengestrol acetate (Imwalle et al., 1998). Progestin treatment mimics the transient rise in P_4 concentration that is observed prior to puberty attainment (Sheffield and Ellicott, 1982), resulting in decreased presence of E_2 receptors on HPG neurons and increased LH concentration and pulse frequency in prepubertal heifers (Anderson et al., 1996; Hall et al., 1997; Imwalle et al., 1998). The success of progestins in inducing puberty varies according to age (Burfening, 1979; Hall et al., 1997) and weight (Gonzalez-Padilla et al., 1975b; Burfening, 1979), indicating that response to progestins depends on the degree to which the HPG has matured prior to exposure (Hall et al., 1997).

Administration of progestins to prepubertal heifers has repercussions on the immediate and future reproductive performance of the herd. Studies have indicated reduced first service conception rates among progestin-exposed heifers in comparison to those naturally attaining puberty (Short et al., 1975; Gonzalez-Padilla et al., 1975b; Burfening, 1979). This is likely a result of the lesser ages and weights of these animals

and the increased incidence of abnormal, short estrous cycles demonstrated by progestin-treated heifers prior to AI (Short et al., 1975). Additionally, from the standpoint of genetic improvement, induction of puberty through use of progestins may mask expression of genetic potential for AP, potentially counteracting attempts to select for reproductive traits (Burfening, 1979).

Calving at two years of age

The challenges of breeding yearling heifers extend beyond the first breeding season. Dystocia is one the largest causes of calf death (NAHMS, 2017) and sources of economic loss in the beef industry (Patterson, 1978). Increased adoption of genetic selection tools for calving ease (Golden et al., 2009) has facilitated a dramatic reduction in incidence of dystocia over time, but rates of dystocia remain greatest among heifers calving at 2 years of age (Laster et al., 1973a; Funnell and Hilton, 2016).

The factors most strongly related to dystocia are birth weight of the calf and the pelvic area (PA) of the dam (Price and Wiltbank, 1978), which are correlated in both size and growth rate to mature weight and breed (Laster, 1974). Growth rate of the pelvic inlet varies throughout the peripubertal period and gestation, with the most rapid periods of growth observed immediately prior to puberty and in the month preceding calving (Bellows et al., 1971; Laster, 1974; Gaines, 1993). Increases in E_2 during puberty attainment and late gestation may be responsible for these observations, as it is plausible that E_2 preferentially increases the growth of flat bones (Adams, 2019). This plausibility is supported by the increased PA observed among heifers exposed to estrogenic growth implants (Staigmiller et al., 1983, Deutscher et al., 1986, Hancock et al., 1994).

A moderate to high degree of heritability has been established for PA, indicating potential for response to selection (Morrison et al, 1986). However, caution is warranted, as selection for greater PA results in indirect selection for greater mature frame size and weight of cows. Correlations between cow size and calf birth weight may consequently increase calf birth weight, inadvertently increasing risk of dystocia (Dawson et al., 1947).

Instead, incidence of dystocia in young dams may be reduced by identifying and removing heifers with extremely small or abnormal pelvises from the herd prior to breeding (Holm et al., 2014). Although all heifers should be bred to bulls expected to produce calves of lighter birth weight to minimize risk of dystocia (Cook et al., 1993), the size and weight of the dam prior to calving is also a significant contributor to incidence of dystocia (Bellows, 1993). Nutrient restriction may limit skeletal growth and prevent adequate growth of the pelvic area prior to calving (Bellows, 1993). However, excessive overfeeding may result in a degree of fatness that restricts the birth canal and increases dystocia (Wiltbank, 1969; Roberts, 1971). Based on these studies, one common management recommendation is that heifers should be fed to achieve 80-85% of their expected mature weight (Hopper, 2021) by the time of calving to ensure appropriate body condition and adequate growth of the pelvic inlet for the passage of a calf.

Postpartum anestrus in the primiparous beef cow

Though the duration of postpartum anestrus is one of the greatest barriers to reproductive efficiency of all cows (Wiltbank, 1970), this challenge is greatest in young primiparous dams. Length of the postpartum interval (PPI) is most influenced by nutrition and suckling of a calf, and the energetic demands for growth and lactation are physiologically prioritized over those for reproduction (Short et al., 1990). Significant

energetic deficits in 2-year-olds that have yet to reach maturity (Morrow et al., 1978) result in longer postpartum intervals, increased incidence of anestrus at the beginning of the second breeding season, and, consequently, poor pregnancy rates (Wiltbank, 1970; Bellows et al., 1982; Goodrich et al., 1985).

Body condition in the periparturient period is the factor with the greatest impact on pregnancy outcomes in the second breeding season (Selk et al., 1988; Spitzer et al., 1995). Postpartum reproductive performance will be optimized when primiparous cows are managed for a BCS of 6 or greater at the time of calving (DeRouen et al., 1994). If this is achieved, moderate changes in nutrient availability or body weight immediately surrounding calving do not impact reproductive performance (Dunn and Kaltenbach, 1980). Substantial pre-calving nutrient restriction, however, may lengthen the PPI, increase the proportion of anestrus animals at the onset of the breeding season, and increase the average days to conception during the breeding season (Dunn et al., 1969; Bellows and Short, 1978). Reproductive performance of heifers that are thin at calving is more dependent on postpartum nutrient supply than that of heifers calving in adequate condition (Ferrell et al., 1982; Lalman et al., 2000). Cow body condition decreases according to milk production (Morris and Wilton, 1976), which differs by age, weight, and breed (Jeffery et al., 1971). Cows that are larger and/or genetically predisposed to greater milk production are likely to experience greater postpartum weight loss, have longer PPI, and achieve lower pregnancy rates in the second breeding season (Cundiff et al., 1974; Notter et al., 1978; Ferrell et al., 1982).

Several general and nutritional management strategies are designed to shorten the PPI. Postpartum diets that are high in energy may reduce the duration of postpartum

anestrus and increase conception rates (Spitzer et al., 1995; Ciccioli et al., 2003). Early weaning of calves may have the same effect by eliminating the energetic demands of lactation and permitting weight gain prior to the second breeding season (Lusby et al., 1981; Laster et al., 1973b). Biostimulation in the postpartum period has been shown to reduce the PPI and increase the proportion of animals expressing estrus early in the breeding season (Gifford et al., 1989; Fernandez et al., 1993).

An alternative to reducing the PPI is to account for the greater time required for primiparous cows to resume cyclicity by intentionally allotting heifers a greater interval between calving and the second breeding season. Management strategies that can accomplish this include breeding heifers in advance of the cow herd and reducing the number of late-calving heifers by restricting the length of the first calving season (Lalman et al., 1997).

Evaluation of Replacement Heifer Candidates

Regardless of whether heifers are raised or purchased, many factors must be considered in selecting replacement females. Reproductive efficiency over the lifetime of a cow is largely influenced by the reproductive outcomes of the first breeding season. As this requires attainment of puberty, evaluations designed to predict AP or assess pubertal status can be valuable.

Genetic merit

Heifer selection based on phenotypic traits is common in commercial production settings. However, characteristics such as weight or conformation may be highly influenced by management and may not be indicative of reproductive maturity.

Evaluation of genotypic merit for reproductive traits may provide an objective prediction of potential fertility, and modern genomic tools can directly assess genetic composition of the animal (Snelling et al., 2012). Pedigree-based indication of genetic merit may be derived from performance data of related animals and is commonly included in expected progeny differences. Unfortunately, development of genetic selection tools for reproductive performance is slow due to the low heritability of reproductive traits, difficulty in identifying and defining measurable traits, and the length of time required for generation of performance data (Cammack et al., 2009).

Management often masks genetic potential for reproduction (Cammack et al., 2009). Furthermore, interaction between multiple maternal traits, such as that observed between milk production and the postpartum interval (Graves et al., 1968), may increase difficulty in determining the genetic merit of the individual traits (Martin et al., 1992). A heifer's genetic potential for AP is expressed prior to the potential for interaction with fertility traits expressed later in life, reducing variation attributable to management or environment to some degree (Martin et al., 1992). Age at puberty attainment is, however, genetically correlated to fertility traits such as yearling (-0.67) or lifetime (-0.76) pregnancy rate (Morris et al., 2000), making it an attractive trait to target for improvement of genetic merit in reproduction (Morris et al., 2000). Direct genetic selection for AP is precluded by the lack of specific genomic markers identified as predictive of puberty attainment (Snelling et al., 2012). However genetic improvement in AP may be achieved by selecting individual animals within a breed or implementing crossbreeding systems.

Determining pubertal status

Evaluation of pubertal status involves use of diagnostic techniques to assess reproductive maturity. Estrous cyclicity may be confirmed by identifying a CL via transrectal palpation or ultrasonography (Hanzen et al., 2000). Functionality of the CL may be directly assessed via measurement of circulating P_4 concentration. Additionally, productive capacity may be estimated by considering morphologic and ultrastructural characteristics of size (Kastelic et al., 1990) and texture (Watson and Munroe, 1980) or via advanced ultrasound techniques such as calculation of pixel value (Singh et al., 1997) or Doppler measurement of luteal blood flow (Lüttgenau et al., 2014).

Palpation is a sensitive technique for CL detection when compared to ovarian dissection (Dawson, 1975; Pieterse et al., 1990) or measurement of serum P_4 concentrations (Boyd and Munro, 1979; Ribadu et al., 1994). However, accuracy in palpating forming and regressing corpora lutea is much poorer than accuracy in identification of mature corpora lutea (Ott et al., 1970; 1986, Sprecher et al., 1989). Ultrasound can be used to detect the CL with nearly complete accuracy when compared to ovarian dissection (Pierson and Ginther et al., 1987). Though ultrasound is most precise for detecting mature corpora lutea, accuracy in identifying forming and regressing structures is not reduced by the same magnitude for ultrasound as for palpation (Sprecher et al., 1989). CL visualization via ultrasound is likely possible on all days of the estrous cycle (Pierson and Ginther, 1987), as the morphological longevity of a single CL exceeds the duration of its functional capacity (Watson and Munro, 1980; Pieterse et al., 1990; Ribadu et al., 1994; Siqueira et al., 2009). Detection via measurement of circulating P_4 concentration, is only possible from approximately the fourth to seventeenth day of the

estrous cycle (Stabendfeldt et al., 1969; Adeyemo and Heath, 1980), making multiple concentration measurements separated by an interval of approximately 10-days necessary for confirmation of pubertal status via this method (Perry et al., 1991).

The circulating P_4 concentration that should be considered indicative of puberty attainment is debated as basal levels may vary considerably with biological type and external influence (Cooke and Arthington, 2009). A concentration of 1.0 ng/ml is a commonly used threshold (Perry et al., 1991) based on the assumption that P_4 levels among heifers lacking corpora lutea are likely to remain below this level (da Rosa and Wagner, 1981). However, concentrations greater than 1.0 ng/ml have been identified in prepubertal heifers experiencing stress induced adrenal P_4 production (Cooke and Arthington, 2009) and circulating P_4 levels below detection have been observed in heifers with confirmed corpora lutea at multiple time points across the estrous cycle (Burke et al., 2001, Cooke and Arthington, 2009). When determining pubertal status, type I errors may be minimized by consideration of circulating P_4 at threshold concentrations of 1.5 – 2.0 ng/ml (Cooke and Arthington, 2009). However, priority is more often placed on minimizing type II errors by considering threshold concentrations of 0.5 ng/ml as indicative of puberty attainment (Busch et al., 2007). Though this may reduce type II errors to some degree, ultrasound confirmation of CL presence at two separate time points is the most effective method of mitigating risk of type II error in determining pubertal status (Kastelic et al., 1990a). Even so, difficulty in identifying the small, embedded corpora lutea of initial estrous cycles (Berardinelli et al., 1979) or the forming and regressing corpora lutea of pubertal heifers may result in type II error via ultrasound (Pieterse et al., 1990).

Characterization of other aspects of ovarian and uterine maturity may inform predictions of proximity to puberty attainment (Anderson et al., 1991). Ovarian follicular activity may be observed via ovarian dissection (Rajakoski, 1960), palpation (Pieterse et al., 1990), or ultrasound (Pierson and Ginther, 1988). Sensitivity for classification of follicles less than 10 mm in diameter by size may be poor via palpation (Pieterse et al., 1990). Ultrasound, however, can be used to accurately detect follicles as small as 2 millimeters, measure follicle size, and visualize the ovary in cross section (Pierson and Ginther, 1988). Ultrasound is also a minimally invasive method for sequential monitoring of follicular structures over the course of an estrous cycle (Pierson and Ginther, 1988) in comparison to the original technique of repeated surgical excision and replacement of ovaries in live animals (Dufour et al., 1972; Matton et al., 1981).

Uterine characteristics vary throughout the estrous cycle in response to shifting hormone concentrations (Pierson and Ginther, 1987). Uterine palpation allows assessment of size and detection of hormonally induced changes in contractility and tone, defined as “the thickness of the digitally compressed uterine walls or the resistance of the myometrium” (Bonafos et al. 1995). Ultrasound may be employed to monitor uterine thickness, edema, or echotexture (Fissore et al., 1986, Pierson and Ginther et al., 1987). Uterine horn diameter can be measured by positioning the ultrasound transducer 2 centimeters cranial to the bifurcation of the uterine body (Heppelmann et al., 2013). It should be noted that accuracy of ultrasound measurements may be influenced by positioning of the uterus within the pelvic cavity (Szenci et al., 2021).

Pre-breeding evaluation

These diagnostic techniques provide pragmatic tools to evaluate reproductive maturity. The techniques used, information collected, and interpretation of data should reflect the purpose of the evaluation relative to animal age and proximity to first breeding (Anderson et al., 1991). Assessment of all heifers 45-60 days prior to breeding may provide a survey of reproductive maturity and facilitate adjustments in management and nutrition to hasten puberty. Assessment 30 days prior to breeding may aid in identifying heifers with a low probability of conception and facilitate culling decisions or selection of an appropriate estrus synchronization program. Interpretative tools have been developed to assist in objectively considering measurable data.

Reproductive tract scoring (RTS) is an accurate method to predict the interval until puberty attainment (Anderson et al., 1991; Holm et al., 2009), pregnancy rate to artificial insemination (AI) (LeFever et al., 1986, Pence et al., 1999), and days to conception within the breeding period (Holm et al., 2015). A single score indicative of reproductive maturity is assigned following transrectal palpation of the uterus and ovaries (Anderson et al., 1991). Heifers with infantile uterine development and pea-sized, undeveloped ovaries are assigned a score of 1 and considered prepubertal. Heifers with poorly developed uteri and very few ovarian follicles are assigned a score of 2, considered peripubertal, and are estimated to be closer to puberty attainment than those with a score of 1. Heifers with moderately uterine development and several follicles in the 8–10-millimeter range are assigned a score of 3; these heifers are also considered peripubertal but estimated to attain puberty within 30 days of evaluation. Heifers assigned scores of 4 and 5 are both considered pubertal. A score of 4 is assigned based on

perception of characteristics indicative of estrus including coiling and tone in the uterus and a preovulatory follicle greater than 10 millimeters in size on one or both ovaries. The presence of a CL and a well-developed uterus results in assignment of a score of 5.

Reproductive tract scoring is considered repeatable within and between technicians (Rosenkrans et al., 2003). However, repeatability is greater for identification of pubertal status than assignment of individual scores (Rosenkrans et al., 2003), as evaluations performed at a single time point reflect both reproductive tract maturity and day of the estrous cycle (Holm, 2006; Holm et al., 2015). Score category may be uncertain when ovarian maturity does not align with uterine maturity based on the individual score definitions (Holm, 2006). In these instances, ovarian score is typically prioritized based on an assumption that uterine assessment is more subjective than ovarian assessment (Rosenkrans and Hardin, 2003; Holm et al., 2016).

Pre-breeding evaluations may integrate predicted proximity to puberty with assessment of additional traits important to reproductive success in the first year or over the lifetime of the cow. Pelvic area is most appropriately used to reduce incidence of dystocia through identification and removal of heifers with the concerningly small pelvises (Basarab et al., 1993; Holm et al., 2014). The size of the pelvic inlet may be determined by using an internal caliper to measure the width at the widest point between the ileal shafts and the height between the pubic symphysis and the sacrum (Deutscher, 1987). Repeatability within and between veterinarian is moderate, but inexperienced technicians are likely to overestimate or underestimate PA (van Donkersgoed et al., 1993; Paputungan et al., 1993). Measurements must be considered relative to puberty attainment and the expected time span until parturition as pelvic size is influenced by

pubertal status (Bullock and Patterson, 1995) and will continue to increase approaching calving (Gaines et al., 1993). Pelvic area and RTS may predict reproductive failure more accurately together than individually (Holm et al., 2016).

Antral follicle count is not related to age at puberty (Mossa et al., 2013; Cushman et al., 2014), but has been suggested as an indicator of fertility and longevity (Ireland et al., 2008). Some evidence shows that animals with lesser AFC have reduced pregnancy rates (Cushman et al., 2009; Mossa et al., 2012; Martinez et al., 2016), later calving dates (McNeel et al., 2015), and greater postpartum interval (Martinez et al., 2016) than those with greater AFC. Despite marked variation between individuals, AFC evaluation via ultrasound is highly repeatable within and between technician, across follicular waves of a single estrous cycle, and between separate estrous cycles (Burns et al., 2005; Cushman et al., 2009; Silva-Santos et al., 2014). Antral follicle count may alternatively be determined by measurement of a growth factor produced exclusively by the granulosa cells of growing follicles known as anti-Müllerian hormone (Weenen et al., 2004; Ireland et al., 2008). As anti-Müllerian hormone concentrations vary little throughout the estrous cycle, a single test may replace ultrasound evaluation for determining AFC in cattle (Ireland et al., 2010; Batista et al., 2014).

Systems integrating a variety of growth and reproductive traits have been developed for different purposes. Stockton et al. (2013) used a regression model to create an index based on age, nutrition, and weight trait measurements of heifers and their dams. The “Maturity Index” is intended to predict breeding maturity and facilitate economically efficient management via selection of homogenous animals or individualization of nutrition for animals differing in requirements. Monday et al. (2018) sought to develop a

practical tool for classifying heifer breeding readiness based on weight, BCS, reproductive tract development, and pelvic size. The "Ready-Intermediate-Problem Matrix" is intended to improve long-term reproductive efficiency of the herd by predicting reproductive performance in the first breeding season. These newer systems may provide useful information when applied in the appropriate context and for their intended purpose but have yet to be widely adopted in commercial production settings.

Conclusion

Economic efficiency in raising beef cattle is predicated on the identification and implementation of management strategies that maximize profit rather than animal performance or production (Miller et al., 2001). However, cost factors have a greater influence on profit than any production or management factors (Miller et al., 2001). In heifer development, profitability will be greatest when optimizing reproductive performance to overall costs of development.

The reproductive performance of heifers bred as yearlings does not exceed that of heifers bred at greater ages when measured by pregnancy rate (Lusby et al., 1979), number of calves weaned (Bernard et al., 1973; Nunez-Dominiquez et al., 1991), or calf weights (Pinney et al., 1972; Bernard et al., 1973; Cundiff et al., 1973). Despite this, breeding yearling heifers to calve at two years of age is more profitable over the lifetime of the animal because of the lesser development costs incurred and the greater potential number of reproductive years (Goodrich et al., 1985; Nunez-Dominiquez et al., 1991). As feed cost is the factor with the single greatest influence on profit (Miller et al., 2001), feeding strategies that maximize the use of inexpensive feedstuffs should be selected to reduce feed costs in the given context (Funston, 2012).

The diversity of feeding strategies implemented for heifer development stems from regional differences in resource availability (Short, 2001, McBride et al., 2011). In extensive development systems such as those based around grazed forages, development to lesser target weights at breeding may offer the greatest profit potential, as cost savings associated with reduced feed inputs are likely to exceed revenue reductions associated with delayed puberty and conception (Creighton et al., 2004; Funston and Deutscher et al., 2004; Martin et al., 2008; Mulliniks et al., 2013; McFarlane et al., 2018). The economic investments in development are greater in systems based on expensive stored feedstuffs; thus, the economic repercussions of poor conception rates are greater in higher input systems, as profit is dramatically reduced by the cost of developing heifers that fail to become pregnant (Lamb et al., 1998). In these more input-intensive systems, reproductive performance may need to be economically prioritized by feeding heifers to achieve greater target weights. In any system, however, capitalizing on compensatory gains during seasons of reduced feed costs may allow overall costs to be reduced without altering reproductive performance (Lynch et al., 1997).

Variation in heifer characteristics like breed, weight, and age is accompanied by variation in the inputs necessary for puberty to be attained (Stockton et al., 2012). The reproductive success of yearling replacements depends on managing so that puberty attainment precedes the start of the first breeding season. The age at which puberty is attained is breed-dependent and correlated to mature cow size (Martin et al., 1992), making it important to match the innate characteristics of heifers to the goals of the system and resources available to accomplish these goals. For this reason, genetic influence on AP should be considered when selecting breeds of sires and dams. Selection

decisions based on genotype may be made prior to birth, but the most profitable stage of development to select replacements based on phenotypic evaluation will vary according to the enterprise's source of revenue, development costs, market for young stock of various ages, and economies of scale (Patterson et al., 2013; Hughes, 2013). All evaluation, selection, and management decisions should be targeted at economically increasing the number of heifers that attain puberty prior to the first breeding season, calve early in the first calving season, and remain in the herd after the second breeding season.

Countless factors may alter the expression of a heifer's genetic threshold for AP, and strategies seek to accelerate the physical, physiologic, and endocrinologic maturation prerequisite to puberty attainment. Methods have been developed to predict AP, evaluate reproductive potential, mitigate the challenges of breeding yearling heifers, and increase reproductive success in the first and subsequent breeding seasons. Ultimately, there is no single formula for profitable heifer development, but economic efficiency can be achieved through careful alignment of animals, resources, management practices, and system goals.

Chapter 2

Implications of disparate uterine and ovarian development observed among heifers evaluated during the peripubertal period

Abstract

Two experiments were conducted to characterize the incidence and implications of disparate ovarian and uterine development during the peripubertal period. Reproductive tract development was assessed, and an individual ovarian score (OS) and uterine score (US) were assigned. Pregnancy outcomes were evaluated on the basis of OS, US and reproductive tract score (RTS: first digit = OS; second digit = US) and relationships between reproductive and physiologic maturity were investigated.

In Experiment 1, duplicate pre-breeding evaluations (PBE) were conducted on 469 heifers approximately 40 and 30 days prior to the start of breeding seasons from 2019-2021. Scores for ovarian (1 = no ovarian development, pea sized; 2 = very few follicles < 8 mm; 3 = several follicles 8-10 mm; 4 = large preovulatory follicle > 10 mm; 5 = corpus luteum present) and uterine (1 = infantile, undeveloped, difficult to palpate; 2 = poorly developed; 3 = distended, moderately developed; 4 = coiled, well-developed, toned; 5 = distended, well-developed) development were assigned following assessment via transrectal palpation and ultrasonography. Uterine horn diameter (UHD), antral follicle count (AFC), largest follicle diameter (LFD), weight, body condition score (BCS), age, and pelvic area (PA) were recorded. Heifers were subjected to the 14-day CIDR-PG estrous synchronization protocol. Split-time artificial insemination was performed and followed by exposure to bulls 14 days later. Pregnancy diagnosis was performed via transrectal ultrasonography.

In Experiment 2, data from 22,173 heifers collected from 2014-2018 were analyzed retrospectively. Pre-breeding evaluations were conducted 35-45 days prior to breeding and scores for ovarian (2=infantile, 3=no significant structures, 4=large follicle and/or corpus luteum) and uterine (2=infantile, 3=mid-sized, distended tract, 4=well-vascularized, distended or coiled tract) development were assigned following assessment via transrectal palpation. Weight, hip height, and PA were recorded, and average daily gain (ADG) was calculated for the development period. Heifers were subjected to the 14-day MGA-PG protocol and artificial insemination (AI) were performed based on detected estrus. Pregnancy diagnosis was performed using transrectal ultrasonography.

The incidence of disparate ovarian score (OS) and uterine score (US) was 33.7% (n = 158/469) in Experiment 1 and 16.3% (n = 3,622/22,174 in Experiment 2. Heifers assigned a RTS of less than 3-3 (Experiment 1 = 4.3% (n = 20/469); Experiment 2 = 0.6% (n = 135/22,174)) demonstrated poor reproductive performance as higher proportions failed to become pregnant in Experiment 1 ($P = 0.03$) and conception to first service AI was decreased in Experiment 2 ($P < 0.01$). Reproductive performance did not differ between heifers assigned a disparate or non-disparate scores of greater than RTS = 3-3. In both experiments, heifers achieving greater physiologic maturity as indicated by age, weight, PA, ADG, or HH exhibited greater reproductive maturity as measured by OS and US, respectively.

We propose that disparate ovarian and uterine development is the result of rapid and asynchronous growth of the reproductive tract during the peripubertal period. Consequently, independent assessment of ovarian and uterine maturity may increase

precision in identifying prepubertal heifers that are unlikely to exhibit satisfactory reproductive performance in the first breeding season.

Introduction

Profitability of cow-calf operations directly depends on the reproductive efficiency and lifetime productivity of the cow. Animals remain in the herd longer and produce more offspring when they calve early in their first calving season, but this requires heifers to attain puberty prior to their first breeding season (Short and Bellows, 1971, Lesmeister, 1973, Cushman et al, 2013). Systems have been developed to evaluate the pubertal status of beef heifers prior to the start of the first breeding season (Anderson et al., 1991, Monday et al., 2019). When compared to inherent traits such as age or physical traits such as body weight, reproductive tract scoring (RTS) is a superior tool for predicting reproductive outcomes (Holm et al. 2009, Pence et al., 1999).

Traditionally, RTS systems incorporate assessment of both uterine and ovarian development into a single score reflecting overall development. In the system described by Anderson et al. (1991), heifers are assigned a score from 1 to 5. Heifers assigned a score of 1 are considered prepubertal. Heifers assigned a score of 2 are considered peripubertal and are estimated to be closer to their first estrous cycle than those with a score of 1. Heifers assigned a score of 3 are also considered peripubertal but with greater proximity to puberty attainment, estimated to exhibit their first estrus within 30 days of evaluation. Heifers are considered pubertal when a large follicle and coiled uterine horns are observed (score 4) or a corpus luteum (CL) is detected (score 5).

Rapid and variable rates of growth of are characteristic of reproductive tract tissues during the peripubertal period (Desjardins and Hafs, 1969). This may be observed as disparate ovarian and uterine development. However, the relative importance of ovarian versus uterine development and the impact of disparate development has yet to be elucidated (Holm, 2006). When dissimilar ovarian and uterine development are perceived, ambiguity in reproductive maturity may raise concerns regarding proximity to puberty attainment and the potential for reproductive success.

The objective of this study was to characterize the incidence and implications of disparate ovarian and uterine development in the peripubertal period. The frequency with which disparities are observed was determined, and reproductive outcomes were assessed relative to score classification. Additionally, the relationship between reproductive and physiologic maturity was investigated by characterizing other physical traits at pre-breeding evaluation and evaluating associations with ovarian and uterine development.

Materials and Methods

Experiment 1

Pre-breeding evaluations (PBE) were conducted on 469 *Bos taurus* heifers at 5 research center locations in the University of Missouri Agricultural Experiment Station network across the spring and fall breeding seasons of 2019-2021. Evaluations of each heifer were conducted in blinded duplicate, both 10 days prior to (PBE1) and coincident with (PBE2) the first treatment of an estrus synchronization protocol.

Pre-breeding evaluations included assessment of reproductive tract development by an experienced technician via a combination of transrectal palpation and transrectal

ultrasonography (SonoSite EDGE equipped with a L52 10.0–5.0 MHz linear-array transducer; SonoSite Inc., Bothell, WA). Scores were assigned for uterine development (1 = infantile, undeveloped, difficult to palpate; 2 = poorly developed; 3 = distended, moderately developed; 4 = coiled, well-developed, toned; 5 = distended, well-developed) and ovarian development (1 = no ovarian development, pea sized; 2 = very few follicles < 8 mm; 3 = several follicles 8-10 mm; 4 = large preovulatory follicle > 10 mm; 5 = corpus luteum present). Uterine horn diameter (UHD) was determined using the digital calipers on the ultrasound and was calculated as the average of the height and width of a cross section of the uterine horn just cranial to the external bifurcation of the uterus. The number of small (3-5 mm), medium (6-10 mm), and large (> 10mm) follicles was recorded for each ovary, and antral follicle count (AFC) was calculated as the sum of all follicles. Largest follicle diameter (LFD), calculated as the average of the height and width measurement, was measured using the digital calipers of the ultrasound for heifers in which the largest follicle observed was greater than 10 mm. Presence and number of corpora lutea observed via ultrasound was recorded for each ovary. Pelvic area (PA) was determined as the product of pelvic height and width as measured with a Rice Pelvimeter (Lane Manufacturing, Denver, CO, USA). Bodyweight and body condition score (BCS; 1–9 scale; 1 = emaciated and 9 = obese) were recorded at the time of evaluation.

Heifers were subjected to the 14-day CIDR-PG estrus synchronization protocol (Leitman et al., 2009). Split-time artificial insemination (AI) was performed (Thomas et al, 2014), followed by exposure to bulls for natural service beginning 14 days after AI. Conception to AI or natural service was determined by measurement of fetal size via ultrasonography (SonoSite EDGE equipped with a L52 10.0–5.0 MHz linear-array

transducer; SonoSite Inc., Bothell, WA) 75-90 days after the start of the breeding season. Failure to become pregnant during the breeding season was confirmed via ultrasonography a minimum of 30 days after the conclusion of the breeding season.

Statistical Procedures

Statistical analyses were performed using SAS (SAS 9.4 Inst. Inc., Cary, NC). Distribution of heifers across RTS category was evaluated (PROC FREQ). Mean and standard deviation of UHD, LFD, age, weight, BCS, AFC, and PA were calculated (PROC MEANS) within ovarian score (OS), uterine score (US), and RTS, respectively.

Correlations of values for UHD, AFC, and LFD at PBE1 and PBE2 were calculated (PROC REG) to determine repeatability between the two time points of evaluation. Correlation matrices were generated (PROC FREQ) for OS and US, respectively, to compare score distributions between PBE1 and PBE2.

Individual logistic regression models were fit (PROC GLIMMIX) to predict conception rate to AI and failure to conceive within the breeding season based on RTS, OS, US, PA, AFC, and UHD when corrected for location and season. Heifers with a RTS of 2-2 were excluded from the analysis of pregnancy outcomes, as the majority of these animals were removed from the herd prior to breeding.

Linear models were fit (PROC GLM) to predict physical traits by RTS, OS, and US, respectively, when corrected for location and season. Physical traits evaluated included age, weight, PA, BCS, UHD, LFD, and AFC. In addition, type III means squares were determined for these models to assess relative contributions of uterine and ovarian score when all other terms were included.

Experiment 2

Data for this experiment were collected at Heartland Cattle Company, (McCook, NE) from 22,174 heifers over five consecutive breeding seasons (2014-2018). Heifers were sourced from 61 independent producers and represented a variety of straightbred and crossbred *Bos taurus* breed compositions.

Heifers arrived at the facility approximately 90 days prior to the beginning of the synchronized breeding season and were fed in individually managed pens to a target pen BCS of 5.75 at synchronized breeding date. Individual weights were taken at the time of initial processing, pre-breeding evaluation, and pregnancy diagnosis. Average daily gain (ADG) was calculated from initial and final body weight values.

Pre-breeding evaluations were conducted 35-45 days prior to the synchronized breeding date. Hip height (HH) was recorded by one of 2-3 individuals per year. Pelvic area was determined as the product of pelvic height and width as measured with a Rice Pelvimeter (Lane Manufacturing, Denver, CO, USA). Reproductive evaluations were performed via transrectal palpation of the reproductive tract by a single veterinarian across the 5-year period. Reproductive tract development was scored using a two-digit scoring system (first digit = ovarian, second digit = uterine) to assess individual ovarian (1 = freemartin, 2 = infantile, 3 = no significant structures, 4 = large follicle and/or corpus luteum) and uterine (1 = freemartin, 2 = infantile, 3 = mid-sized, distended tract, 4 = well-vascularized, distended, or coiled tract) maturity.

All freemartins were removed from the herd at the time of pre-breeding evaluation. Heifers with poor reproductive maturity, conformity to the herd, or

disposition were removed from the development program prior to breeding. Rates of removal prior to breeding by RTS were 2-2 = 95.4% (n = 42/44), 2-3 = 39.5% (n = 15/38), 3-2 = 30.2% (n = 16/53), 3-3 = 10.1% (n = 238/3,941), 3-4 = 4.0% (n = 73/1,832), 4-3 = 2.9% (n = 49/1,698), and 4-4 = 2.0% (n = 293/14,567).

All heifers were subjected to the 14-day MGA-PG estrus synchronization protocol (Brown et al., 1988), and AI was performed by experienced technicians following visual estrus detection. Service sires for AI differed between and within sources, but number of services (1-4) of AI and duration of the synchronized breeding season (35-45 days) varied between sources but was consistent among heifers from a single source.

Pregnancy diagnosis was performed via transrectal ultrasonography by experienced technicians approximately 45 days after the end of the synchronized breeding season. Conception date was determined based on confirmation of fetal size relative to known dates of AI.

Statistical Procedures

Statistical analyses were performed using SAS (SAS 9.4 Inst. Inc., Cary, NC). Distribution of heifers across RTS category was evaluated (PROC FREQ). Mean and standard deviation of pre-breeding weight, PA, ADG, and HH were calculated (PROC MEANS) for each level of RTS.

Individual logistic regression models were fit (PROC GLIMMIX) to compare reproductive tract maturity groups by pre-breeding weight, final weight, PA, HH, and ADG when corrected for year and heifer source prior to development. Models were

restricted by OS category to evaluate uterine maturity or US category to evaluate ovarian maturity.

Individual logistic regression models were fit (PROC GLIMMIX) to predict conception rate to AI and failure to conceive within the breeding season based on RTS when corrected for year and source. Heifers with a RTS of 2-2 were excluded from the analysis of pregnancy outcomes, as the majority of these animals were removed from the herd prior to breeding.

Linear models were fit (PROC GLM) to predict physical traits by RTS, OS, and US, respectively, when corrected for year and heifer source prior to development. Physical traits evaluated included pre-breeding weight, PA, HH, and ADG.

Results

Experiment 1

Distribution of heifers by OS and US is displayed in Table 1. The incidence of disparate US and OS was 33.7% (n = 158/469). Of the total number of heifers, 18.1% (n = 85/469) exhibited a greater OS than US and 22.8% (n = 107/469) exhibited a greater US than OS.

Mean and standard deviation of age, weight, PA, BCS, AFC, and UHD by each RTS category are provided in Table 2. Distribution of age, weight, and pelvic area by RTS, OS, and US is displayed in Figure 1. No significant differences ($P > 0.10$) were identified in UHD, LFD, or AFC between RTS scores. Among heifers with an OS of 3, those with a US of 2 or 3 were younger ($P < 0.001$) than those with a US of 4 or 5. No significant differences ($P = 0.94$) in age were observed among heifers with an OS of 4 or

5. Weight was least ($P < 0.001$) for heifers with a RTS of 2-2. Heifers with a RTS of 3-2 were lighter ($P < 0.001$) than those with a RTS of 3-3, and those with a RTS of 5-3 were lighter ($P < 0.001$) than those with a RTS of 5-5.

When ovarian score is considered independently, an OS of 2 was associated with lesser ($P < 0.001$) age, weight, and PA than an OS of 3, 4, or 5. Age, weight, and PA were also less ($P < 0.001$) among heifers with an OS of 3 than 5 (Figure 1). When uterine score is considered independently, a US of 2 was associated with lesser ($P < 0.001$) age, weight, and PA than a US of 3,4, or 5. Additionally, a US of 3 was associated with lesser ($P < 0.001$) age, weight, and PA than a US of 4 or 5, which were similar to each other. Heifers with a US of 4 had a significantly larger ($P < 0.01$) UHD than those with all other scores.

Independent relationships of OS and US to age, weight, PA, and BCS are displayed in Table 3. A greater amount of variation in age may be attributed to OS than US ($P < 0.001$). A greater amount of variation in weight may be attributed to US than OS ($P < 0.001$). Variation in UHD was attributed to US ($P = 0.002$), but not OS ($P = 0.189$).

Comparison of score frequency between PBE1 and PBE2 is displayed in Table 4 (US) and Table 5 (OS). The US assigned at PBE1 differed from the US assigned at PBE2 for 46.5% (n = 218/469) of heifers, while the OS assigned at PBE1 differed from the OS score assigned at PBE2 for 25.8% (n = 120/469) of heifers. Of these differing scores, 54.6% (n = 119/218) of US differences and 54.2% (n = 65/120) of OS differences exhibited a lesser score at PBE1 and greater score at PBE2. Of heifers with US that differed between PBE1 and PBE2, 21.0% (n = 97/469) were a US of 4 and 5.

The percentage of heifers that were assigned a US of 5 at PBE1 and a US of 3 at PBE2 was 5.5% (n = 26/469). The percentage assigned a US of 3 at PBE1 and a US of 5 at PBE2 was 7.3% (n = 34/469). The percentage assigned a US of 4 at PBE1 and a US of 5 at PBE2 was 9.6% (n=45/469). The percentage of heifers assigned a US of 5 at PBE1 and a US of 4 at PBE2 was 11.1% (n=52/469).

The percentage of heifers assigned an OS of 3 at PBE1 and an OS of 5 at PBE2 or assigned an OS of 5 at PBE1 and an OS of 3 at PBE2 was 5.8% (n=27/469) in both instances. The percentage of heifers assigned an OS of 4 at PBE1 and an OS of 5 at PBE2 was 1.3% (n=6/469). The percentage of heifers assigned an OS of 5 at PBE1 and an OS of 4 at PBE2 was 1.5% (n=7/469).

The repeatability of continuous variables of ovarian and uterine development across the estrous cycle was poor. Correlations between measurements collected at PBE1 and PBE2 were low for UHD ($R = 0.03$) and LFD ($R = 0.01$) for moderate for AFC ($R = 0.40$).

Conception rates to AI as well as the proportion of heifers failing to conceive within the breeding season are presented as a function of RTS (Table 6) or US and OS (Table 7). Days to conception within the breeding season is displayed in Figure 2. Conception rate to AI and days to conception within the breeding season did not differ ($P > 0.10$) by any RTS, OS, or US. The proportion failing to conceive within the breeding season was highest ($P = 0.03$) for heifers with a RTS of 2-3 or 3-2, but no differences were observed between disparate and non-disparate scores of greater values. The proportion of heifers failing to conceive was greater ($P < 0.01$) for those with a US of 2 than those with greater scores and tended to be greater ($P = 0.06$) for those with a US of 3

than those with a US of 5. Additionally, the proportion of heifers failing to conceive during the breeding season was greater ($P < 0.01$) for heifers with an OS of 2 than those with an OS of 4 or 5 and for heifers with an OS of 3 than those with an OS of 5.

Experiment 2

Distribution of heifers by US and OS is displayed in Table 8. The incidence of disparate OS and US was 16.3% (n = 3622/22174).

Mean and standard deviation of weight, PA, ADG, and HH by each RTS category are provided numerically in Table 9 and depicted in Figure 3. Characterization of associations between physical traits and achieving greater uterine or ovarian maturity is depicted in Table 10.

Rates of conception to first service AI and failure to conceive within the breeding season by score are displayed in Table 11. Heifers with a RTS of 2-3 or 3-2 exhibited significantly reduced ($P < 0.01$) conception rates to first service AI compared to those with greater scores. Heifers with disparate RTS of 3-4 and 4-3 did not differ from those with non-disparate RTS of 3-3 or 4-4 ($P > 0.10$).

Discussion

Development of the ovaries and uterus occurs continuously between birth and puberty, with the peripubertal period characterized by rapid development. However, the rate at which maturation occurs is not consistent between these tissues (Desjardin and Hafs, 1969). Observation of disparate ovarian and uterine development is likely the result of this rapid yet asynchronous development of the reproductive tract occurring during the peripubertal period.

Reproductive tract scores are associated with physical traits related to growth and the physiologic maturity of the animal (Anderson et al., 1991, Holm et al., 2009, Montanholi et al., 2008). In Experiment 1, age, weight, and PA differed between RTS (Figure 1). In Experiment 2, achieving greater OS or US was associated with greater weight, PA, ADG, and HH (Table 10). Among heifers with disparate scores in both experiments, greater score values were associated with greater physiologic maturity. For example, PA of heifers with a RTS of 3-3 was less than PA of heifers with a RTS of 3-4 or 3-5 in Experiment 1. This suggests that among heifers with similar ovarian maturity, those identified with greater uterine maturity had achieved greater physiologic maturity. The same conclusion may be drawn for heifers of similar uterine maturity that were identified with greater ovarian maturity. These observations are consistent with the hypothesis that differences in ovarian or uterine maturity observed at the time of evaluation are the result of disparate rates of growth between reproductive tract tissues in the peripubertal period.

The direct relationship observed between RTS and reproductive performance in the present study is consistent with results from previous analysis (Anderson et al., 1991, Pence et al., 1999, Holm et al., 2009, Holm et al., 2015). In Experiment 1, for example, fewer heifers with a RTS of 2-3 or 3-2 became pregnant by the end of the breeding season than those with a RTS of 3-3. Likewise, in Experiment 2, conception rate to AI of heifers with a RTS of 2-3 or 3-2 was less than that of heifers with a RTS of 3-3. However, among heifers with greater score values, those assigned disparate scores (RTS = 3-4, 4-3, 4-5, 5-3, or 5-4) did not differ in conception rate to AI or total pregnancy rates from those assigned non-disparate scores (RTS = 3-3, 4-4, or 5-5) in either experiment.

The similar reproductive performance observed among heifers with disparate and non-disparate scores supports the hypothesis that disparate development is a product of asynchronous growth rather than an indication of any advantage or disadvantage in reproductive development or fertility.

The AFC of individual animals is established prior to puberty (Katska et al., 1984) and has been suggested as an early indicator of inherent fertility and longevity (Ireland et al., 2008). Evidence that AFC did not differ between heifers with disparate or non-disparate scores is consistent with the conclusion that disparate scores are the result of transitional development and not related to inherent fertility. Additionally, the moderate correlation between AFC at PBE1 and PBE2 in Experiment 1 may support previous observation that AFC is repeatable across and between estrous cycles (Burns et al., 2005). The lack of correlation observed between AFC and RTS in this experiment aligns with findings from previous studies to suggest that AFC is not related to age at puberty attainment (Mossa et al., 2013, Cushman et al., 2014).

Among peripubertal heifers, a greater RTS may be interpreted as a greater likelihood of achieving puberty by the time of breeding when pre-breeding evaluations are conducted approximately 30 days prior to the breeding season (Anderson et al., 1991). Pregnancy outcomes of peripubertal heifers with disparate scores depict a continuum of proximity to puberty. For example, heifers with a RTS of 2-3 and 3-3 in Experiment 2 achieved conception rates to AI of 60.9% (n = 14/23) and 68.9% (n = 2,511/3,643), respectively. Similarly, in Experiment 1 a greater percentage of heifers with a RTS of 3-2 failed to become pregnant (77.8%; n = 7/9) than heifers with an RTS of 3-3 (27.5%; n = 33/120) or 3-5 (23.6%; n = 13/55). As the number of heifers that become

pregnant within a defined breeding period is correlated with the number that express estrus early in the breeding season (Short and Bellows, 1971), a plausible explanation for these conception rates is that the proportion of heifers that achieved reproductive maturity prior to or during the breeding season increased by RTS.

The variable rates of growth that characterize reproductive tissue development also characterize growth rates of physical traits such as pelvic area or weight (Laster et al., 1974, Gaines et al., 1993). In the present experiment, the OS and US assigned to heifers were correlated to these physical traits, suggesting that ovarian and uterine evaluation independently detected variation in the true physiologic maturity of heifers. Furthermore, total pregnancy rates differed between individual ovarian and uterine scores. Precision in predicting reproductive outcomes will increase with precision in estimating proximity to puberty attainment. If OS and US are direct indicators of maturity, individually considering ovarian and uterine maturity may increase precision in detecting variation in proximity to puberty attainment among prepubertal and peripubertal heifers.

Previous investigation of ovarian and uterine growth patterns revealed a biphasic pattern of reproductive tissue development in the prepubertal period (Desjardin and Haf, 1969). Studies indicate that ovarian growth largely precedes uterine growth and suggest that uterine development occurs in response to endocrine factors produced by growing ovarian follicles (Honaramooz, 2004). The strong relationship observed between OS and age (Table 3) may indicate that ovarian maturation proceeds with minimal dependence on external factors or managerial influence. Conversely, US exhibits a greater relationship to weight and BCS than age (Table 3), suggesting that uterine development may vary with

management and nutrition to some degree. Though ovarian driven development of the uterus is plausible, the extent to which the uterus lags is likely imperceptible in many cases. Disparities in ovarian and uterine maturity were perceived with relative infrequency as only 33.8% of heifers in Experiment 1 and 16.7% of heifers in Experiment 2 were assigned any form of disparate score. The increased frequency with which disparate development was perceived in Experiment 1 is likely due to increased opportunity for characterizing disparate development with the 1-5 scoring system defined in Experiment 1 compared to the 2-4 system of Experiment 2.

Though the repeatability of RTS within and between evaluators has been established, repeatability across the estrous cycle has yet to be explored (Rosenkrans et al, 2003). Experiment 1 permits consideration of stage-dependent variability by examining differences between blind evaluations conducted 10 days apart at different stages of the cycle. When performed at a single time point, evaluations reflect both the maturity of the reproductive tract and the stage of the estrous cycle. Uterine score takes into consideration factors such as diameter or tone, which vary under the influence of cyclic hormone concentrations (Bonafos et al., 1995). Ovarian score is assigned based on palpation of structures, such as large follicles or corpora lutea, that mature and regress over the course of the estrous cycle (Pierson and Ginther, 1988).

The CL is physically present for a longer period of time than it is functionally producing progesterone (Watson and Munro, 1980, Pieterse et al., 1990, Ribadu et al., 1994, Siqueira et al., 2009). Across the 21-day estrous cycle, progesterone concentrations indicative of CL activity are detectable from approximately the fourth to seventeenth day (Stabendfeldt et al., 1969, Adeyemo and Heath, 1980), while ultrasound visualization of

the CL may be possible on all days (Pierson and Ginther, 1987). However, ultrasound identification of forming and regressing corpora lutea is far less sensitive than identification of the mature CL (Pierson and Ginther, 1987, Pieterse et al., 1990), and corpora lutea formed during the first estrous cycle of heifers are often small, embedded, and difficult to detect (Berardinelli et al., 1979). When determining pubertal status, failure to detect a CL may result in incorrect classification of heifers as prepubertal (Pieterse et al., 1990). Ultrasound may be a more sensitive determinant of pubertal status than measurements of progesterone concentration due to its extended window of detection (Kastelic et al., 1990a). However, misdiagnosis of pubertal status is still likely to occur for some proportion of heifers simply due to stage of the estrous cycle on the day of evaluation.

In Experiment 1, 5.8% (n = 27/469) of heifers were assigned an OS of 5 at PBE1 but an OS of 3 at PBE2. As these heifers could not have truly regressed in ovarian development, it is plausible that the presence of a CL was misdiagnosed at either the first or second evaluation. Similarly, 5.8% (n = 27/469) of heifers were assigned an OS of 3 at PBE1, but an OS of 5 at PBE2. Though misdiagnosis of CL status is a possible explanation for this discrepancy, it is also possible that these heifers experienced their first pubertal ovulation in the 10-day period between evaluations. Further investigation is necessary to determine sources of variation in ovarian classification associated with stage of cycle in peripubertal heifers.

A different US was assigned at PBE1 and PBE2 for 46.5% (n = 220/469) of heifers. However, 21.0% (n = 97/469) of these differences were between scores of 4 and 5. Definitions for uterine scores of 4 and 5 differ only in perception of tone and

distension, both of which are regulated by rising and falling concentrations of estrogen throughout the estrous cycle (Bonafos et al., 1995). Similarly, an OS of 4 is defined by the presence of one or more large follicles, which are only present at specific time points during the estrous cycle (Rajakoski, 1960). The poor repeatability between PBE1 and PBE2 observed for UHD and LFD demonstrates the dependence of these traits on concentrations of circulatory hormones that correspond with stage of the estrous cycle.

Though differences in score classification are to be expected in actively cycling heifers, some differences are likely the product of evaluator subjectivity and the error inherent to evaluating the reproductive tract via palpation (Pieterse et al., 1990). For example, the challenge of perceiving differences as slight as 5 mm in follicular size may lead to misclassifications between an OS of 3 and 4. Similarly, accuracy in assigning a US of 3 or 5 may depend on detecting a 5 mm difference in uterine diameter. A correlation was observed between a US of 4 and UHD, which was significantly larger for these heifers. This provides some degree of confidence that the assigned scores reflected actual variation in uterine characteristics. However, Rosenkrans et al. (2003) indicated that repeatability of reproductive tract scoring is greater for identification of pubertal status than assignment of individual scores, and some distinctions may be inconsequential for predicting reproductive performance. For example, heifers with disparate scores of RTS 4-5 or 5-4 do not differ in maturity but rather stage of the estrous cycle. By classifying all cycling heifers with a score of 4, the RTS system used in Experiment 2 reduces the frequency of disparate scores that represent stage of the estrous cycle rather than a difference in maturity.

Conclusions

Heifers with scores of 3, 4, and 5 in Experiment 1 or scores of 3 and 4 in Experiment 2 did not differ in reproductive outcomes. The potential for evaluator error, similar proximity to puberty attainment, and lack of variation in reproductive outcomes among these heifers precludes the use of RTS as a selection tool for greater fertility or earlier conception within the breeding season (Rosenkrans et al., 2003). Heifers with a RTS of less than 3-3 were least likely to conceive early in the breeding season in this analysis. If RTS were implemented as a screening tool at this cut point it would impact 4.3% (n = 20/469) of heifers in Experiment 1 or 0.6% (n = 135/22,174) of heifers in Experiment 2. When the majority of the herd is suspected to be pubertal, the information provided by RTS may not alter management decisions for a large enough proportion of animals to provide a return on the cost of having pre-breeding evaluations performed. However, when high costs necessitate the use of a lower-input production system and heifers are marginally developed, a sizeable proportion of the herd is likely to be peripubertal. In these systems, RTS may be a valuable tool to identify the heifers least likely to conceive early in the breeding season. When RTS is used for this purpose, independent assessment of uterine and ovarian maturity may increase precision in estimating proximity to puberty attainment.

Systems that lack the ability to differentiate animals based on proximity to puberty attainment may fail to predict differences in pregnancy outcome among peripubertal heifers. For example, the Ready-Intermediate-Problem matrix system was developed as a tool to inform management decisions for improved reproductive performance of the herd (Monday et al, 2018). Though the RIP system considers

additional indicators of growth, such as percentage of mature body weight and pelvic area, assessment of reproductive maturity is simplified by broadly classifying heifers as “Ready”, “Intermediate”, or “Problem”. Evaluation of this system indicated that “Ready” animals are more likely to conceive early in the breeding season than “Problem” animals, but the reproductive performance of “Intermediate” heifers was not significantly different from either “Ready” or “Problem” heifers.

Genetic variation is one of the greatest contributors to variability in the timing of reproductive development of heifers (Cundiff et al., 1986). Though expected progeny differences are a valuable means of evaluating genetic potential, progress in developing tools to aid in the genetic improvement of reproduction is slow due to the low heritability of reproductive traits, difficulty in identifying and defining measurable traits, and the length of time required for generation of performance data (Cammack et al., 2009). Due to the correlation between RTS and age at puberty and its moderate heritability (Anderson et al, 1991), RTS data may be an attractive information source for development of expected progeny differences for reproductive traits. If used in this manner, RTS could indirectly influence the reproductive potential of future generations via improved sire selection. As independent uterine and ovarian assessment is likely to increase precision in estimating proximity to puberty, more detailed scoring systems may have greater success in assessing genetic potential for age at puberty attainment.

The profitability of replacement heifer development is contingent on the enterprise’s source of revenue, development costs, market for animals at various stages of development, and economies of scale (Patterson et al., 2013, Hughes, 2013). When low development costs allow a profit to be generated on non-pregnant heifers, affording

heifers an opportunity to breed with minimal screening or selection may be the most profitable option (Clark et al., 2005). Conversely, when high development costs preclude generation of profit on non-pregnant heifers, RTS may reduce economic loss if used to screen for heifers that are likely to reduce herd profitability by failing becoming pregnant. Reproductive tract scoring should be conducted when the information gained facilitates management decisions that produce economic returns greater than the cost conducting the evaluation.

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Table 1. Distribution of animals by ovarian score (OS) and uterine score (US) in Experiment 1.

		Ovarian Score[1]				
		2	3	4	5	Total
Uterine Score[2]	2	0.6% (3/469)	2.1% (10/469)	0.0% (0/469)	0.0% (0/469)	2.8% (13/469)
	3	1.5% (7/469)	26.4% (124/369)	1.1% (5/469)	3.0% (14/469)	32.0% (150/469)
	4	0.0% (0/469)	1.7% (8/469)	4.1% (19/469)	12.0% (56/469)	17.7% (83/469)
	5	0.4% (0/469)	12.0% (56/469)	0.4% (2/469)	35.2% (165/469)	47.6% (223/469)
	Total	2.1% (10/469)	42.2% (198/469)	5.5% (26/469)	50.1% (235/469)	

[1] Ovarian score: 1 = no ovarian development, pea sized; 2 = very few follicles < 8 mm; 3 = several follicles 8-10 mm; 4 = large preovulatory follicle > 10 mm; 5 = corpus luteum present

[2] Uterine score: 1 = infantile, undeveloped, difficult to palpate; 2 = poorly developed; 3 = distended, moderately developed; 4 = coiled, well-developed, toned; 5 = distended, well-developed

Table 2. Age and physical traits (mean ± SD) of heifers measured at pre-breeding evaluation in Experiment 1.

RTS[1]	Days of Age	Bodyweight (kg)	Pelvic Area (cm^2)	Body Condition Score	Antral Follicle Count	Uterine Horn Diameter (cm)
2-2	385 ± 35 (n = 2)	222 ± 26 (n = 3)	116 ± 21 (n = 3)	4.5 ± 0.9 (n = 3)	15 ± 5 (n = 3)	11.5 ± 1.0 (n = 3)
2-3	411 ± 9 (n = 7)	285 ± 41 (n = 7)	142 ± 5 (n = 7)	4.9 ± 0.5 (n = 7)	12 ± 4 (n = 7)	11.6 ± 1.3 (n = 7)
3-2	425 ± 27 (n = 10)	294 ± 45 (n = 10)	139 ± 15 (n = 10)	5.1 ± 0.6 (n = 10)	13 ± 3 (n = 10)	12.1 ± 1.5 (n = 10)
3-3	436 ± 24 (n = 120)	311 ± 38 (n = 124)	149 ± 13 (n = 124)	5.3 ± 0.5 (n = 123)	17± 7 (n = 121)	12.2 ± 2.0 (n = 124)
3-4	458 ± 20 (n = 8)	353 ± 14 (n = 8)	167 ± 15 (n = 8)	5.7 ± 0.4 (n = 8)	16 ± 6 (n = 8)	13.4 ± 1.1 (n = 8)
3-5	443 ± 25 (n = 56)	340 ± 27 (n = 56)	158 ± 16 (n = 56)	5.6 ± 0.3 (n = 55)	19 ± 9 (n = 55)	13.1 ± 2.0 (n = 54)
4-3	408 ± 27 (n = 2)	276 ± 55 (n = 5)	145 ± 22 (n = 5)	4.8 ± 0.6 (n = 5)	20 ± 5 (n = 5)	11.7 ± 1.8 (n = 5)
4-4	436 ± 29 (n = 19)	316 ± 49 (n = 19)	152 ± 16 (n = 19)	5.1 ± 0.7 (n = 19)	17 ± 9 (n = 18)	12.9 ± 2.1 (n = 19)
4-5	422 ± 27 (n = 2)	356 ± 41 (n = 2)	167 ± 8 (n = 2)	5.5 ± 0.0 (n = 2)	19 ± 4 (n = 2)	12.6 ± 1.5 (n = 2)
5-3	453 ± 19 (n = 14)	315 ± 42 (n = 14)	157 ± 16 (n = 14)	5.5 ± 0.5 (n = 14)	22 ± 10 (n = 14)	12.0 ± 1.0 (n = 14)
5-4	453 ± 18 (n = 55)	349 ± 37 (n = 56)	167 ± 13 (n = 56)	5.5 ± 0.4 (n = 56)	18 ± 7 (n = 55)	13 ± 1.8 (n = 56)
5-5	448 ± 22 (n = 161)	354 ± 37 (n = 165)	167 ± 17 (n = 165)	5.7 ± 0.4 (n = 165)	18 ± 7 (n = 165)	12.7 ± 1.8 (n = 164)

[1] Reproductive tract scores were assigned 30 days prior to the beginning of the breeding season based on assessment of reproductive tract maturity via rectal palpation. The first number of the score indicates ovarian maturity. The second number of the score indicates uterine maturity.

Table 3. Variation in physical traits of heifers attributable to ovarian or uterine score in Experiment 1.

		Mean Square[1]	*P*
Age	Ovarian	3,698.56	< 0.001
	Uterine	1,547.68	0.009
Weight	Ovarian	33,390.41	< 0.001
	Uterine	64,055.48	< 0.001
Pelvic Area	Ovarian	1,421.41	< 0.001
	Uterine	2,342.58	< 0.001
Body Condition Score	Ovarian	1.09	< 0.001
	Uterine	1.73	< 0.001
Uterine Horn Diameter	Ovarian	4.93	0.189
	Uterine	15.45	0.002

[1] Mean squares calculated using type III sum of squares to determine variation in physical traits attributable to ovarian or uterine score, respectively, when all other terms are included.

Table 4. Comparison of uterine score distribution at pre-breeding evaluation 1 (PBE1) and pre-breeding evaluation 2 (PBE2).

		Uterine Score[1] at PBE2				
		2	3	4	5	Total
Uterine Score[1] at PBE1	1	0.2% (1/469)	0.2% (1/469)	0% (0/469)	0% (0/469)	0.4% (2/469)
	2	0.9% (4/469)	4.7% (22/469)	0.4% (2/469)	1.1% (5/469)	7.0% (33/469)
	3	1.7% (8/469)	18.8% (88/469)	1.9% (9/469)	7.2% (34/469)	29.6% (139/469)
	4	0.0% (0/469)	2.8% (13/469)	4.3% (20/469)	9.6% (45/469)	16.6% (78/469)
	5	0% (0/469)	5.5% (26/469)	11.1% (52/469)	29.6% (139/469)	46.3% (217/469)
	Total	2.8% (13/469)	32.0% (150/469)	17.7% (83/469)	47.5% (223/469)	

[1] Uterine score: 1 = infantile, undeveloped, difficult to palpate; 2 = poorly developed; 3 = distended, moderately developed; 4 = coiled, well-developed, toned; 5 = distended, well-developed

Table 5. Comparison of ovarian score distribution at pre-breeding evaluation 1 (PBE1) and pre-breeding evaluation 2 (PBE2).

Ovarian Score[1] at PBE1	Ovarian Score[1] at PBE2				
	2	3	4	5	Total
1	0.2% (1/469)	0.0% (0/469)	0.0% (0/469)	0.0% (0/469)	0.2% (1/469)
2	0.9% (4/469)	2.8% (13/469)	0.6% (3/469)	0.4% (2/469)	4.7% (22/469)
3	1.1% (5/469)	30.1% (141/469)	2.8% (13/469)	5.8% (27/469)	39.7% (186/469)
4	0% (0/469)	3.6% (17/469)	0.6% (3/469)	1.3% (6/469)	5.5% (26/469)
5	0% (0/469)	5.8% (27/439)	1.5% (7/469)	42.6% (200/469)	49.9% (234/469)
Total	2.1% (10/469)	42.2% (198/469)	5.5% (26/469)	50.1% (235/469)	100.0% (469)

[1] Ovarian score: 1 = no ovarian development, pea sized; 2 = very few follicles < 8 mm; 3 = several follicles 8-10 mm; 4 = large preovulatory follicle > 10 mm; 5 = corpus luteum present

Table 6. Pregnancy outcomes of heifers by reproductive tract score[1] (RTS) in Experiment 1.

RTS[1]	Conceived to first AI service		Failed to become pregnant	
	%	Proportion	%	Proportion
2-3	28.6%	2/7	71.4%[a]	5/7
3-2	22.2%	2/9	77.8%[a]	7/9
3-3	51.7%	62/120	27.5%[b]	33/120
3-4	42.9%	3/7	28.6%[b]	2/7
3-5	60.0%	33/55	23.6%[b]	13/55
4-3	40.0%	2/5	20.0%[b]	1/5
4-4	52.6%	10/19	5.3%[b]	1/19
4-5	50.0%	1/2	50.0%[b]	1/2
5-3	71.4%	10/14	21.4%[b]	3/14
5-4	61.8%	34/55	10.9%[b]	6/55
5-5	62.2%	102/164	15.2%[b]	25/164

[a-e] Percentages within column with different superscripts differ ($P < 0.05$).

[1] Reproductive tract scores were assigned 30 days prior to the beginning of the breeding season based on assessment of reproductive tract maturity via rectal palpation. The first number of the score indicates ovarian maturity. The second number of the score indicates uterine maturity.

[2] Conception to first AI service was determined by measurement of fetal size via ultrasonography 75-90 days after the start of the breeding season. Failure to become pregnant during the breeding season was confirmed via ultrasonography a minimum of 30 days after the conclusion of the breeding season

Table 7**.** Pregnancy outcomes of heifers by uterine or ovarian score in Experiment 1.

	Uterine Score[1]				Ovarian Score[2]			
	Conceived to first AI service[2]		Failed to become pregnant[3]		Conceived to first AI service[2]		Failed to become pregnant[3]	
	%	Proportion	%	Proportion	%	Proportion	%	Proportion
2	20.0%	2/10	80.0%[a]	8/10	25.0%	2/8	75.0%[a]	6/8
3	52.1%	76/146	28.8%[b]	42/146	52.4%	100/191	28.8%[a,b]	55/191
4	58.8%	47/80	11.3%[c]	9/80	52.0%	13/25	12.0%[b,c]	3/25
5	61.5%	136/221	17.6%[b,c]	39/221	62.7%	146/233	14.0%[c]	34/243

[a-e] Within column, percentages with different subscripts differ ($P < 0.05$).

[1] Uterine score were assigned 30 days prior to the beginning of the breeding season based on assessment of uterine maturity and tone via rectal palpation.

[2] Ovarian scores were assigned 30 days prior to the beginning of the breeding season based on assessment of ovarian structures via rectal palpation

[3] Conception to first service AI was determined by measurement of fetal size via ultrasonography 75-90 days after the start of the breeding season.

[4] Failure to become pregnant during the breeding season was confirmed via ultrasonography a minimum of 30 days after the conclusion of the breeding season.

Table 8. Distribution of animals by ovarian score and uterine score in Experiment 2.

		Ovarian Score[1]			
		2	3	4	Total
Uterine Score[1]	2	0.2% (44/22,174)	0.2% (53/22,714)	0.0% (0/22,174)	0.4% (97/22,174)
	3	0.2% (38/22,174)	17.8% (3,941/22,174)	7.7% (1,698/22,714)	25.6% (5,677/22,174)
	4	0.0% (1/22,174)	8.3% (1,832/22,174)	65.7% (14567/22,174)	74.0% (16,400/22,174)
	Total	0.4% (83/22,174)	26.3% (5,826/22,174)	73.4% (16,265/22,174)	

[1] Ovarian Score: 1 = freemartin; 2 = infantile, 3 = no significant structures; 4 = large follicle and/or corpus luteum

[2] Uterine Score: 1 = freemartin, 2 = infantile; 3 = mid-sized, distended tract; 4 = well-vascularized, distended, or coiled tract

Table 9. Physical traits (mean ± SD) of heifers measured at pre-breeding evaluation in Experiment 2.

RTS[1]	Bodyweight (kg)	Pelvic area (cm^2)	Average Daily Gain (kg/d)	Hip Height (cm)
2-2	328 ± 50 (n = 44)	172 ± 24 (n = 44)	1.22 ± 0.26 (n = 2)	47.3 ± 1.7 (n = 44)
2-3	328 ± 35 (n = 38)	174 ± 20 (n = 38)	1.40 ± 0.40 (n = 23)	47.1 ± 1.4 (n = 38)
3-2	335 ± 39 (n = 53)	182 ± 18 (n = 53)	1.38 ± 0.30 (n = 37)	47.6 ± 1.6 (n = 53)
3-3	350 ± 33 (n = 3,941)	194 ± 17 (n = 3,941)	1.30 ± 0.33 (n = 3,695)	47.5 ± 1.3 (n = 3,941)
3-4	358 ± 33 (n = 1,832)	198 ± 17 (n = 1,832)	1.29 ± 0.37 (n = 1,757)	47.7 ± 1.3 (n = 1,832)
4-3	359 ± 32 (n = 1,698)	198 ± 17 (n = 1,698)	1.29 ± 0.31 (n = 1,648)	47.8 ± 1.3 (n = 1,698)
4-4	370 ± 33 (n = 14,567)	204 ± 18 (n = 14,567)	1.26 ± 0.33 (n = 14,261)	48.0 ± 1.3 (n = 14,567)

[1] Reproductive tract scores were assigned 30 days prior to the beginning of the breeding season based on assessment of reproductive tract maturity via rectal palpation. The first number of the score indicates ovarian maturity. The second number of the score indicates uterine maturity.

Table 10. Effect of ovarian or uterine maturity on physical traits measured at pre-breeding evaluation in Experiment 2.

	Ovarian[1]		Uterine[2]	
	F-statistic	*P*	F-statistic	*P*
Pelvic Area	94.05	< 0.001	75.57	< 0.001
Hip Height	8.41	0.004	1.91	0.167
Pre-breeding Weight	0.95	0.33	13.72	<0.001
Final Weight	11.55	0.001	5.45	0.02
Average Daily Gain	10.18	0.001	8.90	0.003

1 Models for ovarian maturity were restricted to animals with uterine score of 3 and ovarian score of 4 was predicted by pre-breeding weight, final weight, PA, HH, and ADG.

[2] Models for uterine maturity were restricted to animals with an ovarian score of 3 and uterine score of 4 was predicted by pre-breeding weight, final weight, PA, HH, and ADG.

Table 11. Pregnancy outcomes of heifers by reproductive tract score[1] (RTS) in Experiment 2.

RTS[1]	Conceived to first AI service		Failed to become pregnant	
	%	Proportion	%	Proportion
2-3	60.9%[a]	14/23	17.4%[a,b]	4/23
3-2	62.2%[a]	23/37	13.5%[a,b]	5/37
3-3	68.9%[b]	2,511/3,643	14.4%[a,b]	526/3,643
3-4	69.1%[b,c]	1,215/1,759	12.2%[a]	213/1,749
3-5	66.7%[b,c]	1,100/1,649	13.7%[b]	226/1,649
4-3	69.6%[c]	9,938/14,274	13.9%[b]	1,990/14,274

[a-e] Within column, percentages with different subscripts differ ($P < 0.05$).

[1] Reproductive tract scores were assigned 35-45 days prior to the synchronized breeding date based on assessment of reproductive tract maturity via rectal palpation. The first number of the score indicates ovarian maturity. The second number of the score indicates uterine maturity.

[3] Conception to first service AI or failure to become pregnant was determined by measurement of fetal size via ultrasonography 45 days after the end of synchronized breeding

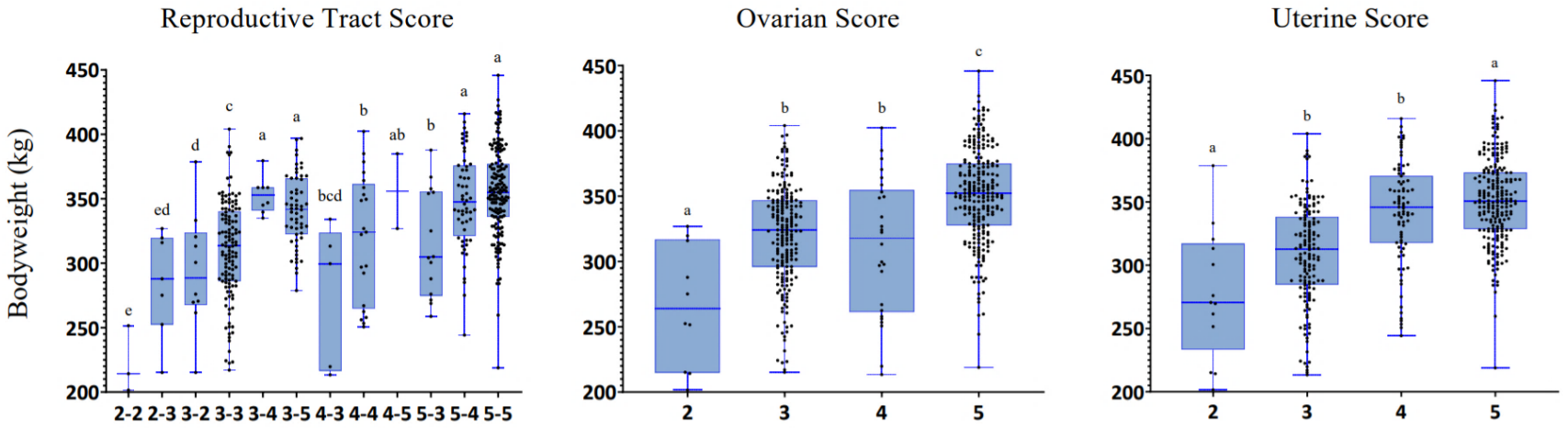

Figure 1.1. Box and whisker dot plot showing bodyweight in kilograms (kg) of heifers in Experiment 1 when grouped by reproductive tract score (RTS), uterine score (US), or ovarian score (OS). All scores were assigned 30 days prior to the beginning of the breeding season based on assessment of reproductive tract maturity via rectal palpation. Ovarian scores reflect ovarian size and presence of ovarian structures. Uterine scores reflect uterine size and tone. Reproductive tract scores are the combined OS (first digit) and US (second digit) of an individual heifer. Bodyweight in kilograms (kg) was measured 30-45 days prior to the beginning of the breeding season. Black points represent individual heifers. Blue boxes span the interquartile range with the median represented by the middle line. Minimum and maximum values are represented by the vertical blue bars. Different letters indicate a difference in mean values between score groups ($P < 0.05$).

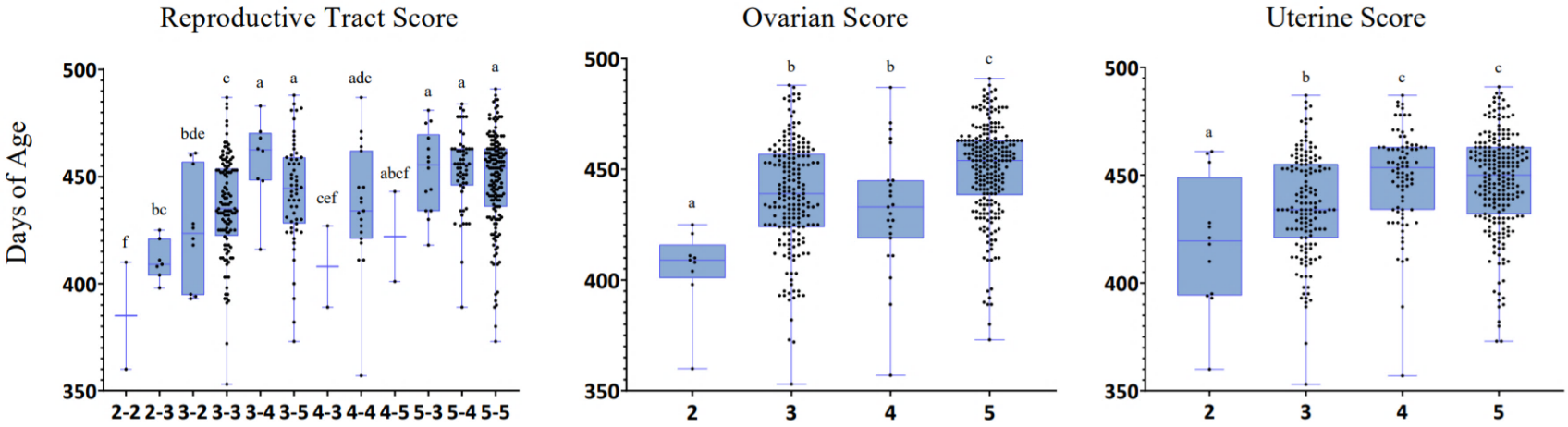

Figure 1.2. Box and whisker dot plot showing days of age of heifers in Experiment 1 when grouped by reproductive tract score (RTS), uterine score (US), or ovarian score (OS). All scores were assigned 30 days prior to the beginning of the breeding season based on assessment of reproductive tract maturity via rectal palpation. Ovarian scores reflect ovarian size and presence of ovarian structures. Uterine scores reflect uterine size and tone. Reproductive tract scores are the combined OS (first digit) and US (second digit) of an individual heifer. Days of age was determined at the time of pre-breeding evaluation. Black points represent individual heifers. Blue boxes span the interquartile range with the median represented by the middle line. Minimum and maximum values are represented by the vertical blue bars. Different letters indicate a difference in mean values between score groups ($P < 0.05$).

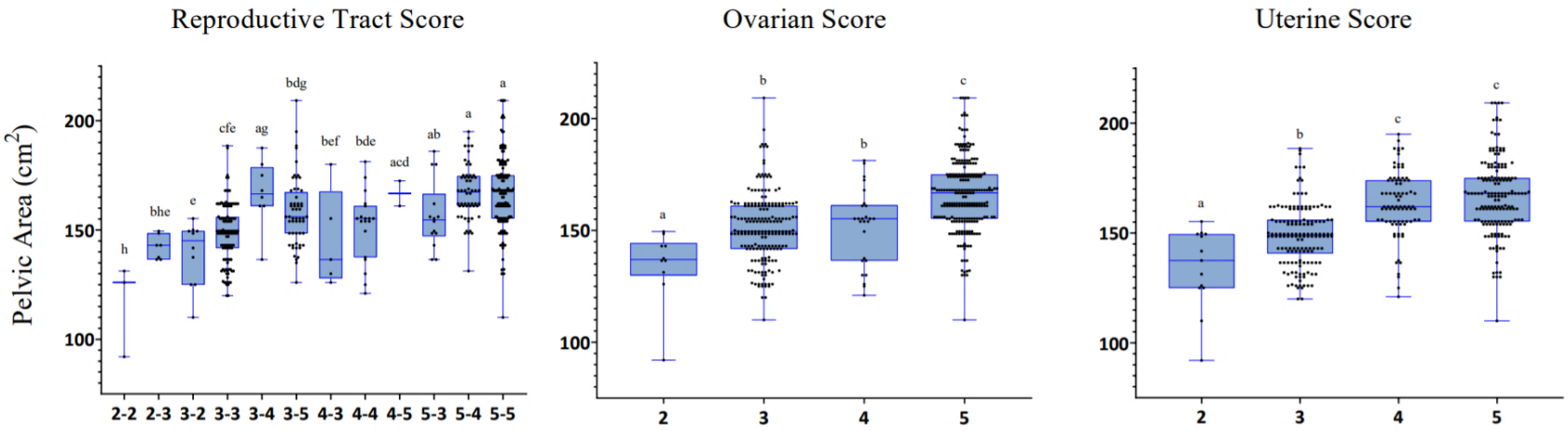

Figure 1.3. Box and whisker dot plot showing pelvic area of heifers in Experiment 1 when grouped by reproductive tract score (RTS), uterine score (US), or ovarian score (OS). All scores were assigned 30 days prior to the beginning of the breeding season based on assessment of reproductive tract maturity via rectal palpation. Ovarian scores reflect ovarian size and presence of ovarian structures. Uterine scores reflect uterine size and tone. Reproductive tract scores are the combined OS (first digit) and US (second digit) of an individual heifer. Pelvic area (PA) in square centimeters (cm2) was calculated as the product of pelvic height and width as measured with a Rice Pelvimeter. Black points represent individual heifers. Blue boxes span the interquartile range with the median represented by the middle line. Minimum and maximum values are represented by the vertical blue bars. Different letters indicate a difference in mean values between score groups ($P < 0.05$).

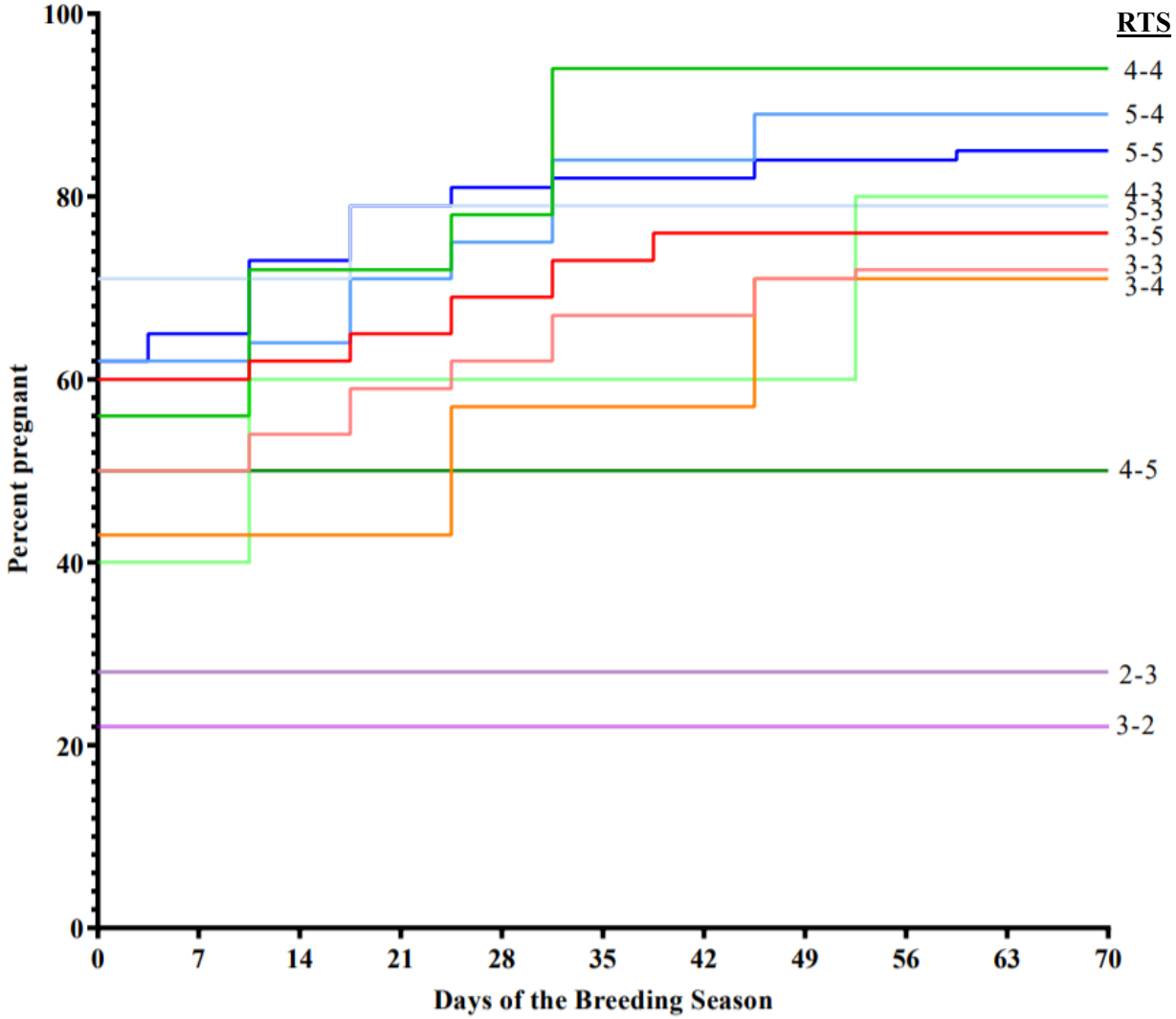

Figure 2. Days to conception within the breeding season by reproductive tract score category. Reproductive tract scores were assigned 30 days prior to the beginning of the breeding season based on assessment of reproductive tract maturity via rectal palpation. The first number of the score indicates ovarian maturity. The second number of the score indicates uterine maturity. All heifers were subjected to the 14-day CIDR-PG estrous synchronization protocol. Artificial insemination was performed on Day 0. Date of conception was determined by measurement of fetal size via ultrasonography 75-90 days after the start of the breeding season.

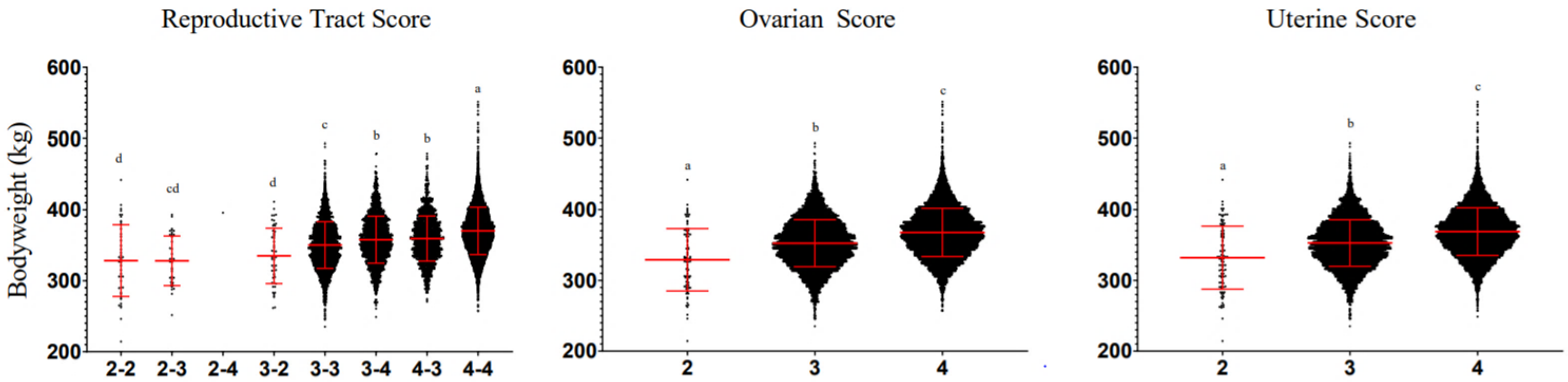

Figure 3.1. Scatter plot showing variation in weight of heifers in Experiment 2 when grouped by reproductive tract score, uterine score, or ovarian score. All scores were assigned 35-45 days prior to the synchronized breeding date based on assessment of reproductive tract maturity via rectal palpation. Ovarian scores reflect ovarian size and presence of ovarian structures. Uterine scores reflect uterine size and tone. Reproductive tract scores are the combined ovarian score (first digit) and uterine score (second digit). Bodyweight in kilograms (kg) was measured 35-45 days prior to the synchronized breeding date. Black points represent individual heifers. Mean values are represented by the middle blue bar. Standard deviation is represented by the vertical bars. Different letters indicate a difference in mean values between score groups ($P < 0.05$).

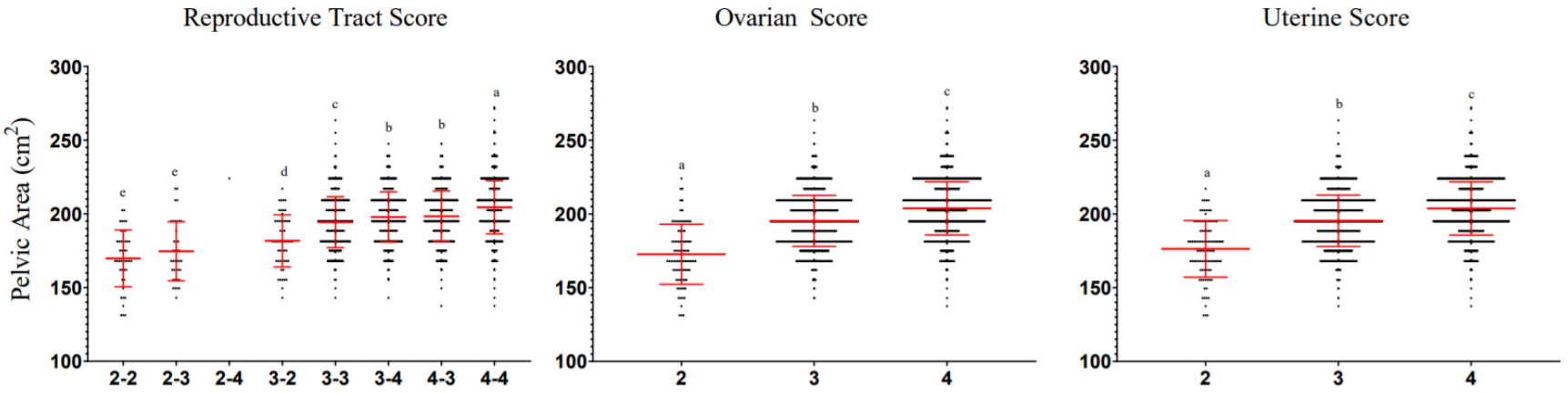

Figure 3.2. Scatter plot showing variation in pelvic area (cm^2) of heifers in Experiment 2 when grouped by reproductive tract score, uterine score, or ovarian score. All scores were assigned 35-45 days prior to the synchronized breeding date based on assessment of reproductive tract maturity via rectal palpation. Ovarian scores reflect ovarian size and presence of ovarian structures. Uterine scores reflect uterine size and tone. Reproductive tract scores are the combined ovarian score (first digit) and uterine score (second digit). Pelvic area in square centimeters (cm2) was calculated as the product of pelvic height and width as measured with a Rice Pelvimeter.Black points represent individual heifers. Mean values are represented by the middle blue bar. Standard deviation is represented by the vertical bars. Different letters indicate a difference in mean values between score groups ($P < 0.05$).

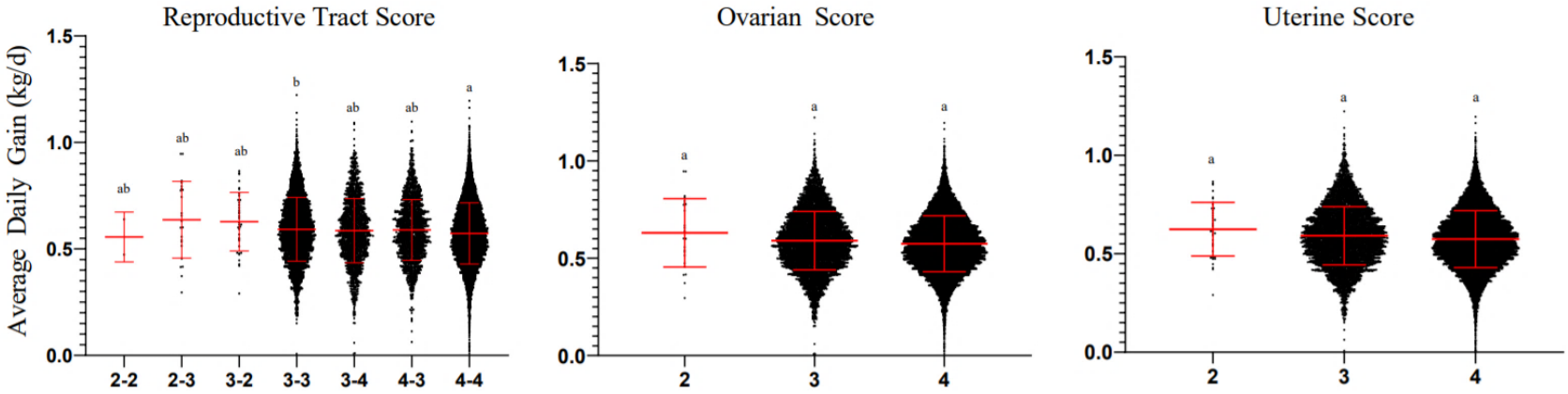

Figure 3.3. Scatter plot showing average daily gain (ADG) of heifers in Experiment 2 when grouped by reproductive tract score, uterine score, or ovarian score. All scores were assigned 35-45 days prior to the synchronized breeding date based on assessment of reproductive tract maturity via rectal palpation. Ovarian scores reflect ovarian size and presence of ovarian structures. Uterine scores reflect uterine size and tone. Reproductive tract scores are the combined ovarian score (first digit) and uterine score (second digit). Average daily gain calculated by dividing the weight in kilograms gained during the development period by the number of days from the beginning of the development to pregnancy diagnosis. Black points represent individual heifers. Mean values are represented by the middle blue bar. Standard deviation is represented by the vertical bars. Different letters indicate a difference in mean values between score groups ($P < 0.05$).

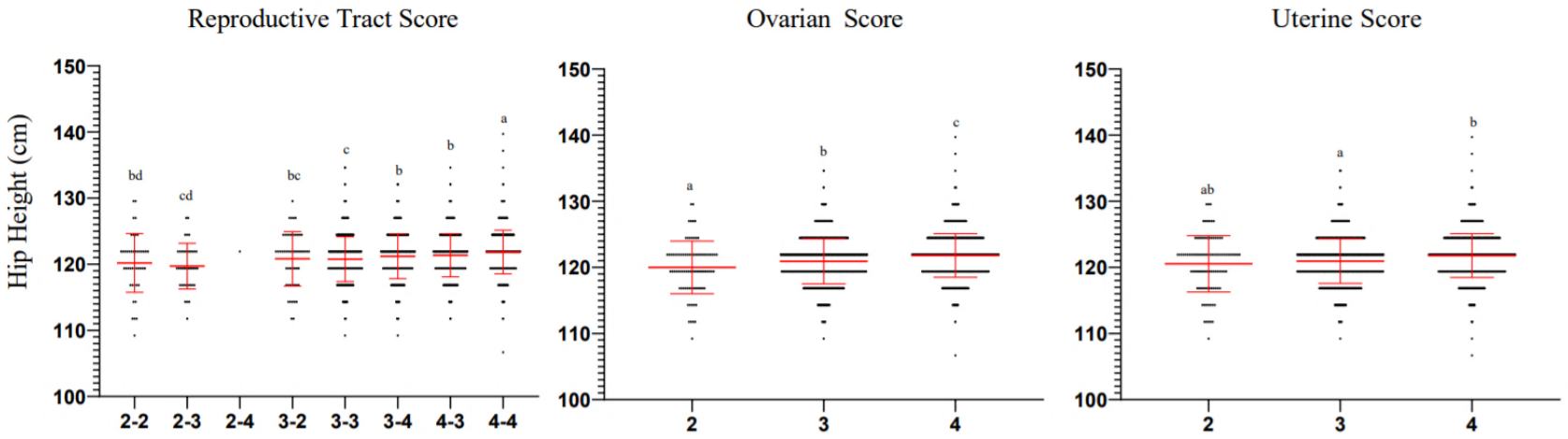

Figure 3.4. Scatter plot showing variation in hip height (cm) of heifers in Experiment 2 when grouped by reproductive tract score, uterine score, or ovarian score. All scores were assigned 35-45 days prior to the synchronized breeding date based on assessment of reproductive tract maturity via rectal palpation. Ovarian scores reflect ovarian size and presence of ovarian structures. Uterine scores reflect uterine size and tone. Reproductive tract scores are the combined ovarian score (first digit) and uterine score (second digit). Hip height was measured as height in centimeters (cm) at the hooks of the animal 35-45 days prior to the synchronized breeding date. Black points represent individual heifers. Mean values are represented by the middle blue bar. Standard deviation is represented by the vertical bars. Different letters indicate a difference in mean values between score groups ($P < 0.05$).

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Vita

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