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Oocyte production in the early postpartum cow

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OOCYTE PRODUCTION IN THE EARLY POSTPARTUM COW

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
In partial fulfillment of the
Requirements for the degree of
Doctor of Philosophy

in

The Interdepartmental Program
in Animal and Dairy Sciences

by
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ABSTRACT

The postpartum period has been the focus of numerous studies; however, there is no information available relating to oocyte production in early postpartum cows. Ovaries of early postpartum cows were stimulated with FSH to produce follicular development and oocytes. The objectives of these studies were: (1) to evaluate the use of FSH for oocyte production in early postpartum beef cows, (2) to evaluate follicular response and oocyte quality of beef cows treated with FSH shortly after calving, (3) to evaluate FSH for oocyte production in early postpartum dairy cows, (4) to evaluate responses of FSH-treated, early postpartum beef cows in a transvaginal ultrasound-guided oocyte aspiration (TUGA) experiment using polyvinylpyrrolidone as the vehicle for FSH in a single dose plus energy supplementation and/or bovine somatotropin treatment, and (5) to evaluate if a single dose of GnRH could modify LH secretion in beef cows at day 5 and day 30 postpartum. In each of 4 experiments, TUGA was used to harvest oocytes from the FSH-stimulated donor cow ovaries. It was demonstrated that early postpartum cows did respond to exogenous FSH treatment with good follicular development and produced quality oocytes shortly after parturition. Harvested oocytes were subjected to IVF procedures. The number of follicles (>5 mm) aspirated, number of oocytes, recovery rate, number that cleaved, number of blastocysts developing from cleaved embryos, and blastocyst production rate per donor was: 19.4, 8.8, 64%, 4.3, 1.4 and 32.3%, respectively, for cows treated with FSH and oocytes harvested between day 5 and 20 postpartum. The number of follicles aspirated per donor at day 25 and 35 postpartum was 20.4 for the FSH-treated group, resulting in 12.9 oocytes (recovery rate, 63%). It was determined that a single FSH injection could stimulate the ovaries of the early postpartum cow as early as day 10 postpartum with a follicular response of 17.5 follicles and 11 oocytes recovered per cow (recovery rate, 69%). Furthermore, live calves were obtained from harvested oocytes from FSH-treated cows in the early postpartum period. In summary, TUGA should be considered as an alternative tool for obtaining oocytes for embryo production from early postpartum cows.
INTRODUCTION

The postpartum period has been studied extensively because of its importance in the economic outcome of cattle operations. The main characteristic of this physiological state is a period of acyclic infertility immediately after parturition (Callahan et al., 1971; Short et al., 1990; Zollers et al., 1991; Roche et al., 1992; Schrick et al., 1993; Breuel et al., 1993a; Inskeep, 1995). To restore postpartum fertility, a chain of physiological events has to occur, and depending on these events the cow could resume normal-length estrous cycles. Hormonal and ovarian events must have a mutual synchrony to re-establish female cyclicity after calving.

The postpartum period in the cow is characterized by a low frequency of pulsatile LH (Echternkamp and Hansel, 1973; Arije et al., 1974; Gauthier et al., 1982; Walters et al., 1982a; Williams et al., 1983; Peters and Lamming, 1986; Jagger et al., 1987; Nett et al., 1988). This secretory LH pattern changes as the time when the first ovulation is about to occur (Rawlings et al., 1980; Webb et al., 1980).

Although the hypothalamus contains sufficient amounts of GnRH to stimulate release of LH from the anterior pituitary, its release is inhibited during the early postpartum period in the cow (Moss et al., 1985). With a change in pituitary sensitivity, ovarian follicular activity is restored during the final portion of the postpartum period (Walters et al., 1982b; Williams et al., 1982; Schallenberger et al., 1984; Peters et al., 1985).

Exogenous GnRH can be used to increase ovarian activity and ovulation in anestrous postpartum cows (Gillian et al., 1981; D’Occhio et al., 1989; Crowe et al., 1993); however, the corpus luteum (CL) that is produced usually has a short life span (Riley et al., 1981; Butcher et al., 1992).

GnRH has been widely used to: 1) promote ovarian activity, 2) induce an LH surge and 3) induce ovulation. A single injection of GnRH increases the LH surge and has caused ovulation in dairy cows between day-10 to day-18 postpartum (Britt et al., 1974; Kesler et al., 1977, 1978; Fernandes et al., 1978; Foster et al., 1980; Schallenberger et al., 1984). In beef cows, the same response is usually found between day-21 to day-31 postpartum (Smith et al., 1979; Carter et al., 1980; Irvin et al., 1981; Pratt et al., 1982; Wettemann et al., 1982; Troxel et al., 1983, 1984). Correspondingly, the ovaries have also been the primary focus of studies on infertility in the postpartum cow. There have been attempts to induce superovulation with FSH in postpartum dairy cows (Kaminura et al., 1996) with ovulation occurring; however, embryos produced in cows before day-30 postpartum have had lower viability (Schrick et al., 1993). Breuel et al. (1993b) has suggested that these postpartum embryos could be affected by uterine or environmental factors. To avoid uterine influence on these embryos, transvaginal ultrasound-
guided oocyte aspiration should be considered as an alternative tool to obtain oocytes from these postpartum females.

The transvaginal oocyte recovery technique was first described in cows in the late 1980s (Pieterse et al., 1989). This assisted reproductive technology has proven to be a reliable procedure for obtaining oocytes from live cows (Callessen and Christenson, 1987; Pieterse et al., 1990; Kruip et al., 1994; Looney et al., 1994). Transvaginal ultrasound-guided oocyte aspiration has been successfully applied in humans (Lenz et al., 1981; Dellenbach et al., 1985), cattle (Pieterse et al., 1989, 1991a, 1991b, 1992; Walton et al., 1993; Looney et al., 1994; Hill et al., 1998), horses (Brück et al., 1992; Cook et al., 1992; Brück et al., 1994; Meintjes et al., 1995b), prepubertal calves (Duby et al., 1996), goats (Graff et al., 1999), sows (Bellow et al., 2001), buffalo cows (Boni et al., 1996) and other exotic species (Adams et al., 1991; Asa et al., 1998).

Transvaginal ultrasound-guided oocyte aspiration (TUGA) from live cows in combination with in vitro maturation (IVM) in vitro fertilization (IVF) and in vitro culture (IVC) can be a source of IVF-derived embryos in cattle. Using this technology, oocytes could be obtained from cows at different physiological stages, such as cycling cows (Kruip et al., 1991), pregnant cows (Meintjes et al., 1995a) and nonreproductive problem donor cows (Looney et al., 1994). Bovine oocytes obtained with the TUGA and subjected to IVF developed into embryos that have sufficient quality to establish and maintain a pregnancy when transferred to recipient females (Gibbons et al., 1995).

This researcher feels that this approach may provide an alternative reproductive method as an efficient source of oocytes for the production of IVF embryos before 30 days postpartum. The application of this procedure in postpartum cows together with IVM, IVF and IVC might have a potential for producing extra embryos and calves when the postpartum cow is not able at this time.

Therefore, the purpose of this study was: 1) to evaluate FSH treatment for oocyte production in early postpartum beef cows, 2) to evaluate follicular development and oocyte quality of beef cows treated with FSH shortly after calving, 3) to evaluate the effect of FSH for oocyte production in early postpartum Holstein dairy cows, 4) to evaluate the response of FSH-treated early postpartum beef cows in a transvaginal ultrasound-guided oocyte aspiration program using polyvinylpyrrolidone as the vehicle for the FSH in a single dose along with energy supplementation and/or bovine somatotropin treatments and 5) to evaluate if a single GnRH challenge would modify LH secretion at both day-5 and day-30 postpartum beef cows.
CHAPTER I
LITERATURE REVIEW

Physiology of the Postpartum Beef Cow

The postpartum cow has been the subject of numerous research reports over the years. Increased research interest in the postpartum period occurred in the 1920s (Short et al., 1990). Of primary interest, researchers attempted to understand the complexity of physiological processes starting immediately after calving. One classic report outlined the key factors associated with the interval from parturition to first estrus in beef cattle (Warnick, 1955).

After parturition, cows are acyclic for variable periods of time. Several factors contribute to this infertile period, such as uterine involution (Perkins and Kidder, 1963; Morrow et al., 1966; Moller, 1970; Kindahl et al., 1992, 1999), short estrous cycles (Odde et al., 1980; Butcher et al., 1992), anestrous and general infertility (Short et al., 1990).

The postpartum period is characterized by involution of the uterus and re-establishment of ovarian function, with the main purpose being to prepare the animal for a new pregnancy. Uterine involution results from three overlapping processes: contraction, loss of tissue and tissue repair (Gier and Marion, 1968; Kindahl et al., 1999; Yavas and Walton, 2000a). Using ultrasonography, it was confirmed that uterine involution in dairy cows was completed by ~40 days after calving (Okano and Tomizuka, 1987).

Short estrous cycles prevent fertility during the first 20 days after parturition by causing the cow to return to estrus before pregnancy recognition occurs (Short et al., 1990). The duration of the postpartum anestrus is affected by four major factors: season, nursing, nutrition and cow age (Roche et al., 1992; Yavas and Walton, 2000b).

Resumption of Follicular Development

Follicular development in cattle has been under intensive study. The wave pattern of follicular development involves a group of antral follicles in a cohort that are available for gonadotropin treatment. Each wave is composed of a dominant follicle and subordinate follicles that originate from the same follicular cohort (Perry et al., 1991). Initially, Rajakosky (1960) suggested that there are 2 waves of follicular growth in heifers, one between days 1 and 12 and the second between day 13 and ovulation. Later, two, three and four follicular waves were described in cycling cows (Sirois and Fortune, 1988; Fortune et al., 1991; Fortune, 1993; Rhodes et al., 1995; Ginther et al., 1996; Adams, 1999).

After parturition, follicular development involves growth and regression of follicles without ovulation. Follicular growth resumes at various intervals after calving in both dairy and beef
cattle. The pattern of resumption of follicular activity in the postpartum period of dairy and beef cows is presented in Figure 1.1.

This follicular development resumes early after calving in both beef and dairy cows with the first dominant follicle (DF) detected morphologically around day-10 to day-15 postpartum (Murphy et al., 1990; Savio et al., 1990; Roche et al., 1992). In beef cows the first dominant follicle develops within the first 15 days postpartum. In contrast, dairy cows develop the first DF within 10 days postpartum; however, most of these follicles undergo atresia.

Follicles are classified according their diameter as small-sized (<5 mm), medium-sized (6 to 9 mm) and dominant follicles (>10 mm) (Lucy et al., 1993; Kirby et al., 1997a). The size of small follicles increases in dairy cows as the days postpartum increase (Wagner and Hansel, 1969). Before day-25 postpartum, the average number of small follicles decreased; whereas, the number of large follicles increased with increasing days postpartum. Apparently, follicles in smaller classes move into the larger classes with increasing days postpartum. The number of medium-size follicles remains unchanged, likely because this represents a transitory size class.

In dairy cows, medium-size follicles (5-10 mm in diameter) are detectable between day-5 to day-15 postpartum and the first ovulation usually occurs 15 to 30 days postpartum (Morrow et al., 1966; Wagner et al., 1969; Callahan et al., 1971; Kesler et al., 1978, 1979; King and Macleod, 1984; Murphy et al., 1990; Beam and Butler, 1997; Yavas et al., 1999).

In contrast, beef cows develop medium-size follicles after 5 days postpartum and the first ovulation occurs ~25 days postpartum (Stevenson and Britt, 1979; Crowe et al., 1993; Stagg et al., 1995; Crowe et al., 1998; Yavas et al., 2000b). The DF of both dairy and beef cows usually fails to ovulate during the early postpartum interval (Spicer et al., 1986a, 1986b; Stagg et al., 1995). Follicular waves do not occur before 5 days postpartum in either beef or dairy cows (Murphy et al., 1990; Stagg et al., 1995; Crowe et al., 1998).

The interval to detection of the first postpartum DF was reported to be 9.6 ± 0.6 days in beef cows and the number of DF before the first ovulation was reported to be 2.1 ± 0.6 (Crowe et al., 1998). In dairy cows, the interval to detection of the first postpartum DF was found to be 10.2 ± 2.2 and the first ovulation occurred ~20.4 days postpartum (Morrow et al., 1966). The emergence of a wave is also associated with a surge in FSH concentrations in cycling cows (Adams et al., 1992). The emergence of each postpartum follicular wave was preceded by a 2 to 4 day rise in circulating FSH concentrations (Crowe et al., 1998). There is follicular development in both ovaries of postpartum beef cows; however, postpartum follicular activity in the ovary ipsilateral to the previously gravid uterine horn was reported to be lower than that in the contralateral ovary (Nation et al., 1999).
Figure 1.1. Proposed scheme of resumption of ovarian cycles during the postpartum period in dairy and beef cows (Savio et al., 1990; Roche et al., 1992).
Nutrition and Follicular Development in Postpartum Beef Cows

Nutrition is one of the major factors that affect the duration of the postpartum period. The effects of nutrition have been most commonly measured using energy as a variable. These effects are considered more important when feeding animals before calving than those effects after calving (Short et al., 1990).

Energy balance increases medium-size and preovulatory follicles but not small follicles with increasing days postpartum (Lucy et al., 1990). Follicles between 5 to 7 mm in diameter were found to be unaffected by dietary energy levels before and after parturition. Growth of follicles after calving has been shown to be affected by energy intake (Wettemann and Bossis, 1999). Perry et al. (1991) showed that high energy diets before and after parturition had fewer medium-size follicles than high energy diets given before a low energy diet postpartum. In contrast, Ryan et al. (1992) reported that the development of medium-size follicles was increased with high energy diets. This high energy diet also increased total cholesterol and progesterone levels in follicular fluid (Ryan et al., 1992; Hawkins et al., 1999).

Body condition score (BCS) affects the postpartum period at different levels. Low body condition scores (<4) have a negative influence on reproduction than a BCS of 7. Postpartum differences in diets have their primary effect when BCS at calving is <6 and when the feed intake was less than 100% of the NRC requirement (Short et al., 1990).

Ryan et al. (1994) showed that BCS was related to the number of medium- and large-size follicles from day-7 to day-17 postpartum. Cows with a BCS of 8 had more medium- and large-size follicles than cows with BCS of 3 (very thin), 4 (thin) or 6 (optimal). Cows with BCS 8 had more large follicles than cows with BCS of 3 or 4 at 9 days postpartum (Wettemann and Bossis, 2000). Therefore, nutrition intake has a major influence on activation of the ovaries in the postpartum period in the beef cow (Spitzer et al., 1995).

Stephan and Butler (1997) found that higher fat diets increased the diameter of the DF in the early postpartum period and at moderate level of supplementation, resulted in higher a peak of estradiol during the first follicular wave and a shorter anovulatory interval. The initiation of follicular development postpartum, including recruitment and selection of a dominant follicle, occurred regardless of metabolic status and in response to factors unrelated to energy metabolism. In this study, the plane of nutrition did not affect the growth of medium-size and large-size follicles. Therefore, failure of the DF to mature and ovulate may be responsible for prolonged postpartum intervals in beef cows.
Hypothalamic-Pituitary-Ovarian-Axis in the Postpartum Cow

Resumption of estrous cycles in the postpartum cow is accomplished by a complex relationship among the hypothalamus, the pituitary and the ovary (Short et al., 1990). This relationship starts during the late prepartum period where steroid hormones, such as progesterone (P₄) and estradiol 17-β (E₂) concentrations are elevated and FSH and LH are at low levels (Crowe et al., 1998). The high circulating concentrations of these steroids inhibit secretion of GnRH from the bovine hypothalamus (Nett, 1987b; Yavas and Walton, 2000b). This results in the accumulation of pituitary FSH, suppression of FSH release (Crowe et al., 1988) and, in most cases, depletion of pituitary LH stores (Nett, 1987).

GnRH content in the preoptic area, media basal or median eminence of ewes was not changed from day 1 after parturition until the animals began cycling ~40 days postpartum (Moss et al., 1980). Similarly, the GnRH content in the same area of the hypothalamus has been found not to change in beef cows from day-5 to day-30 postpartum (Moss et al., 1985). Carruthers et al. (1980) found no differences in the GnRH content between suckling and nonsuckling cows. Moreover, there is evidence that the GnRH content in the hypothalamus of the postpartum cows was twice the amount found in cycling cows (Nett, 1987). These observations suggest that during the postpartum period the hypothalamus contains a sufficient amount of GnRH to stimulate the anterior pituitary (Nett et al., 1987); however, the number of receptors for GnRH in the pituitary anterior was lowest immediately following parturition, between day-1 to day-5 postpartum. The GnRH receptors increased on day-15 postpartum and then gradually declined through day 45 postpartum (Nett, 1987; Nett et al., 1988). Likewise, Moss et al. (1985) found a reduced number of GnRH receptors at day-20 and day-30 postpartum compared with those on day-5 and day-10 postpartum.

FSH and LH Content or Release

Mean serum concentrations of FSH in cows were found not to be different before and after parturition (23.8 and 25.2 ng/mL, respectively) (Crowe et al., 1998). An emergence of a follicular wave within 5 days postpartum is associated with a previous 2- to 4-day elevation in FSH in the early postpartum period (Crowe et al., 1998). This FSH content in the anterior pituitary decreases after parturition (Wagner et al., 1969). Nett et al. (1988) showed that the pituitary content of FSH in cows changed over the postpartum period. However, FSH pulses remain constant with fluctuations equivalent to those found in cyclic cows (Lamming and MacLeod, 1988; Crowe et al., 1998).

The pituitary content of LH in cows increases from low levels prepartum and immediately postpartum to higher levels at day-10 and day-20 days postpartum (Lamming et al., 1981). This
content in the anterior pituitary is lowest on day-1 postpartum and remains low through day-15 postpartum. Pituitary LH content increases by around day-30, remaining constant to day-45 after calving (Nett, 1987).

The pituitary content of LH is reduced by 95% during gestation in cows and ewes and then LH content gradually increases after parturition (Nalbandov and Casida, 1940). In suckling cows, the pituitary content of LH increases from very low levels at parturition to levels similar to those present in cycling animals by around day-30 postpartum (Moss et al., 1985). These observations suggest that if pulses of GnRH were released into the hypophyseal portal system, GnRH would be lower than those found in cycling cows. Therefore, it was suggested that lack of stores of LH in the pituitary gland, rather than reduced sensitivity to GnRH, was one of the initial limitations to the resumption of normal estrous cycles in the postpartum period (Nett, 1987). The low pituitary LH content was possible due to high circulating concentrations of estrogens during late gestation (Nett et al., 1988).

Plasma concentrations of LH in suckling and milked cows were 0.08 and 0.9 ng/mL, respectively, by day-1 to day-5 postpartum. These LH concentrations increased gradually from 0.8 ng/mL to 1.2 ng/mL through day-21 to day-30 postpartum (Lamming et al., 1981). In beef cows, LH levels after parturition varied between 0.5 to 2.0 ng/mL the first 21 days postpartum (Arije et al., 1974). Humphrey et al. (1983) found similar low values in the early postpartum cow, within the first 10 days at 0.7 ng/mL and 0.4 ng/mL at 30 days postpartum. The low serum concentration of LH is caused by a low frequency pulsatile secretion pattern of LH. The pulse pattern of LH had very low frequency (<1 pulse/4-hour) (Short et al., 1990). This pulse pattern increases the LH frequency and concentration in cattle prior to the first pre-ovulatory LH surge (Arije et al., 1974; Carruthers et al., 1980; Walters et al., 1982a; Nett et al., 1988).

Low plasma LH concentrations after parturition are followed with an increase in basal secretion and the development of a pulsatile pattern. This LH secretion appears earlier in dairy than in beef cows. The development of a frequent pulsatile pattern of LH secretion occurs between day-13 to day-20 in dairy cows (Peters et al., 1981); whereas, this development occurs between day-25 and day-32 in postpartum beef cows (Rawlings et al., 1980; Lamming et al., 1981). Thus, a pulsatile LH pattern is a prerequisite for the onset of ovarian cycles in the postpartum period (Peters et al., 1981; Humphrey et al., 1983).

**Progesterone and Estradiol Release**

Plasma (P4) concentrations in cows decrease abruptly ~24 hours prior to parturition from values of 6 ng/mL (Arije et al., 1974) to values of 0.5 to 3 ng/mL at calving (Stabenfeldt et al., 1970). After parturition, the progesterone levels decline to low or undetectable levels until the
The initiation of the corpus luteum formation after the first ovulation (Echternkamp and Hansel, 1973).

Plasma (E$_2$) concentrations have been reported to be elevated at parturition (67.9 pg/mL) compared with 1 to 4 days postpartum in dairy cows (8.7 pg/mL) (Echternkamp and Hansel, 1973). In beef cows, E$_2$ concentrations have been reported to be 113 pg/mL on the day of parturition, decreasing to 7 pg/mL at day-6 postpartum and remaining at this level until just before the onset of estrus (Humphrey et al., 1983). The increase in E$_2$ after parturition is thought to be due to developing follicles during the first follicular wave of the postpartum interval (Arije et al., 1974; Humphrey et al., 1983; Crowe et al., 1988). Estradiol increments have been positively correlated with circulating LH concentrations in beef and dairy cows (Kesler et al., 1979).

**Effect of Postpartum Nursing**

The inhibitory influence of suckling on resumption of postpartum ovulatory cycles in cattle has been researched extensively. Suckling is a primary stimulus in controlling reproductive cycles after parturition in female mammals (Edgerton, 1980). Suppression of cyclic ovarian activity is the primary outcome in the suckled beef cow. Suckling inhibits the release of GnRH from the hypothalamus (Acosta et al., 1983; Hinshelwood et al., 1985; Williams, 1990). The inhibitory effect of suckling on returning to estrus is documented in dairy cows (Oxenreider and Wagner, 1971) and in beef cows (Radford et al., 1978). Thus, the suckling stimulus increases the time to first estrus by increasing the sensitivity of the hypothalamus to the negative feedback of E$_2$ during the postpartum period, resulting in decreased LH secretion (Acosta et al., 1983).

The hypothalamic content of GnRH is thought not to be affected by suckling status; however, GnRH concentrations in the hypophyseal portal system are suppressed by suckling (Carruthers et al., 1980; Nett et al., 1988). This hypothalamic content is not believed to change during the postpartum period in suckled cows (Nett, 1987b) and in suckled and nonsuckled dairy cows (Carruthers et al., 1980).

As a result, the LH pattern is absent to promote the final process of the follicular development (Silviera et al., 1993). This absence of LH pulses is due to depletion of LH stores in the anterior pituitary and is independent of suckling (Nett, 1987; Nett et al., 1987; Williams, 1990). Weaning immediately after parturition does not initiate LH pulses and ovulation. Several researchers showed that pituitary stores of LH are replenished between day-7 to day-20 postpartum in dairy cows (Carruthers et al., 1980) and after day-20 in beef cows (Carter et al., 1980; Odde et al., 1980; LaVoie et al., 1981; Yavas and Walton, 2000b). Following the
replenishment in LH stores, the LH pulses become dependent on the absence of the suckling effect (Nett, 1987).

Replenishment of the LH stores in the anterior pituitary increases the frequency of LH pulses (and subsequent ovulation) when there is a complete, temporary or partial weaning in beef cattle (Acosta et al., 1983; Edwards, 1985; Houngh ton et al., 1990; Griffith and Williams, 1996). Thus, the physiological effect of suckling in cows is due to suppressing the pulsatile release of LH from replenished pituitary LH stores by inhibiting GnRH discharges from the hypothalamus during the postpartum period (Carruthers et al., 1980; Lamming et al., 1981; Walters et al., 1982; Hinshelwood et al., 1985; Yavas and Walton, 2000a).

Another important factor in the control of GnRH release from the hypothalamus in the postpartum cow is that endogenous opioids likely participate in the regulation of GnRH and LH secretion in the early postpartum period (Whisnant et al., 1986). Suppression of GnRH has been attributed to the suckling-stimulated release of endogenous opioids (Gregg et al., 1986; Nett, 1987). This opioid inhibition of LH decreases as the postpartum interval increases (Whisnant et al., 1986).

**Short Cycles During the Postpartum Period**

Morrow et al. (1996) recently reaffirmed the occurrence of a short luteal phase following first ovulation in the postpartum period of cattle. The first ovulation postpartum generally occurs with silent estrus and is followed by a short estrous cycle of 8 to 12 days of duration in the majority of cows (King and McLeod, 1984; Savio et al., 1990; Stagg et al., 1995; Yavas et al., 1999).

Occurrences of short estrous cycles frequently appear during the first 30 to 40 days postpartum in beef cows (Short et al., 1990). The oocyte released during this short estrous cycle in beef cattle can be fertilized (Short et al., 1990). However, pregnancy is not maintained, apparently because the corpus luteum is regressing before the ovary receives the uterine signal that a pregnancy exists (Odde et al., 1980; Ramirez-Godinez et al., 1982). Short cycles are also common after induced ovulation in the postpartum period by weaning, weaning plus GnRH injection, a single injection of GnRH, intermittent injection of GnRH and continuous infusion of GnRH as well as after the first ovulation at puberty (Gonzalez-Padilla et al., 1975; Foster et al., 1980; Smith et al., 1983; Troxel et al., 1984; Jagger et al., 1987; Breuel et al., 1993a).

Premature release of prostaglandin \( F_{2\alpha} \) (PGF\(_{2\alpha}\)) from the uterus on day 5 of a short estrous cycle is probably the mechanism involved in subnormal luteal function in sheep and cattle (Zollers et al., 1991). Similar conclusions were obtained when premature release of PGF\(_{2\alpha}\)
The CL that is formed during a short cycle is smaller and secretes less P₄ than a CL during a normal cycle (Lishman et al., 1979; Rutter and Randel, 1984). Luteal function can be obtained during an early postpartum period by pre-treatment with a progestin (Ramirez-Godinez et al., 1981; Troxel et al., 1993). This process involves a reduction in prostaglandin production.
from the uterus, with this effect mediated by the ovary rather than pituitary (Garverick et al., 1988; Short et al., 1990). Pre-treatment with norgestomet (17\(\alpha\) acetoxy-11\(\beta\) methyl-19-nor-pregn-4-ene-3,20 dione), which is a progestin with high progesterational potency, has been used as a pre-treatment in postpartum cows to increase the proportion of cows that formed a CL with a normal-length life span (Sheffel et al., 1982).

**Induction of Ovulation in Postpartum Cows**

Anestrus during the postpartum interval is primarily due to a failure of ovulation of dominant follicles rather than a delay in the development of follicular waves during the postpartum period. Follicular development resumes soon after parturition; however, the dominant follicles do not undergo the final maturation process and thus do not ovulate. The lack of ovulation of the early dominant follicle is due to an inadequate LH peak (Murphy et al., 1990; Roche et al., 1992; Crowe et al., 1993; Stagg et al., 1995; Williams and Griffith, 1995). In the early postpartum period this LH deficiency is mainly due to depletion of this hormone in the anterior pituitary. Pituitary stores of LH in cows are replenished between day-15 to day-30 postpartum (Yavas and Walton, 2000b).

There are various methods that have been attempted to either initiate resumption of cyclicity or induce ovulation. These methods have been conducted to increase the LH pulse frequency, such as removing the suckling stimulus by weaning (Faltys et al., 1987), inducing ovulation of dominant follicles by exogenous GnRH (Gillian et al., 1981) or estradiol injection (Peters et al., 1984b; Garcia-Winder et al., 1988) and increasing the LH pulse frequency by exogenous progestins (Thompson et al., 1999).

Weaning has been considered one of the nonhormonal methods to increase the LH pulse frequency in postpartum cows. Complete weaning, temporary weaning and partial weaning increase the release patterns of GnRH (Gazal et al., 1998) as well as frequency in LH pulses (Walters et al., 1982c; Whisnant et al., 1986; Faltys et al., 1987). One important factor to consider in weaning is that LH needs to be replenished to produce an LH peak. Furthermore, weaning increases follicular receptor concentrations for LH and consequently then ovulation (Walters et al., 1982a, 1982b, 1982c). The presence of short cycles has been reported after weaning (Odde et al., 1980; Breuel et al., 1993a).

Another classic method to induce an LH peak in mammals is the introduction of a male into the female population. In mice, the Whitten effect and the Bruce effect demonstrated that the male effect (olfactory effect) could induce a preovulatory surge of LH and ovulation (Turner, 1966). In sheep the ram effect initiates cyclicity (Cushwa et al., 1992). Other species such as
goats, sows, and cows exhibit similar behavioral responses (Alberio et al., 1978; Ott et al., 1980; Walton, 1986); however, the use of this method to alter LH output is less practical in cattle.

**Inducing Ovulation with GnRH**

GnRH is a decapetide that regulates both LH and FSH release in most mammals (Schally et al., 1971). GnRH has been characterized and used to release LH in various species including ewes (Gonzalez and Murphy, 1998), calves (Rodriguez and Wise, 1991) and postpartum cows (Fonseca et al., 1980).

A single injection of GnRH, intermittent injections of GnRH (in a pulsatile pattern) and continuous infusion of GnRH have been widely used to increase the frequency of LH pulses and ovulation during the postpartum period in cattle. Most of these GnRH protocols have been used after day-20 postpartum; however, very few have been reported for LH levels following a GnRH injection in the early postpartum period. Yavas and Walton (2000a) have recently reviewed GnRH protocols used during the postpartum period in beef cattle.

Using a single GnRH injection gives a different response in dairy and beef cattle, which is related to the time when GnRH is given and the LH surge occurs after parturition. In dairy cows, a single injection of GnRH on day-10 to day-18 can cause an LH surge and ovulation (Britt et al., 1974; Fernandes et al., 1978; Kesler et al., 1978; Foster et al., 1980). In contrast, a single injection of GnRH induced the same LH response but after 20 days postpartum in beef cows (Echternkamp, 1978; Carter et al., 1980; Kesler et al., 1980; Troxel et al., 1980; Pratt et al., 1982; Wetteman et al., 1982; Williams et al., 1982; Smith et al., 1983; Troxel et al., 1984; Dunn et al., 1985). Other researchers have reported that cows did not respond to a single injection of GnRH before 20 days postpartum (Webb et al., 1977; Fonseca et al., 1980).

Two research reports indicated that cows did respond to a single injection of GnRH at day-5 postpartum (Carter et al., 1980; Williams et al., 1982). Carter et al. (1980) reported a LH peak of 3.2 ng/mL in GnRH-treated beef cows compared with <1 ng/mL in control cows at day-5 postpartum. Furthermore, calf removal did not increase the LH release at day-5 postpartum in beef cows. In this experiment, concentrations of LH were similar between suckled and nonsuckled cows and in cows that did not ovulate.

Williams et al. (1982) proposed that the LH release response after a single GnRH injection was correlated with increasing days postpartum. Cows treated with GnRH at day-3 postpartum responded with a small release of LH. Cows had the highest LH level of 4 ng/mL at ~120 minutes after the GnRH injection. Perhaps, the response of these cows was possible because there were small amounts of releasable LH in the anterior pituitary of those cows shortly after parturition.
A single GnRH injection in Holstein cows induced LH release and ovulation after 14 days postpartum; however, these cows did not have a normal-length estrous cycle until day 65 after parturition (Britt et al., 1974). Short estrous cycles are also the main characteristic following a single GnRH injection in beef cows in the early postpartum period (Pratt et al., 1982).

A similar LH response occurs after a single GnRH injection in both dairy and beef cows (Britt et al., 1974; Fonseca et al., 1980; Troxel et al., 1980). The highest values were found ~2 hours after the injection and returned to basal concentrations within 9 hours (Wettemann et al., 1982).

Continuous infusion of GnRH has been reported to induce an LH surge and ovulation in postpartum beef cows (Jagger et al., 1987; Lamming and McLeod, 1988; D’Occhio et al., 1989; Lofstedt et al., 1981). Roberts et al. (1989) reported serum LH concentrations of 44 ng/mL in response to intravenous infusion of GnRH (15 µg/hour for 12 hours) at day-35 postpartum in beef cows. A single intramuscular injection of GnRH encapsulated to release 15 µg/day for 30 days increased LH pulse frequency in beef cows at day-5 postpartum; however, short estrous cycles were detected in all females that were infused with GnRH (Jagger et al., 1987; Roberge et al., 1992).

Intermittent injections of GnRH have been used to try and mimic endogenous GnRH pulses. The intermittent injections of GnRH induced pulses of LH from 2.8 to 3.3 ng/mL (Walters et al., 1982c; Spicer et al., 1986a). Moreover, Edwards et al. (1983) suggested that pulsatile injections of GnRH at ~day-30 postpartum could not re-activate ovarian cyclicity; however, cows did respond with a small LH release. The same pattern of short estrous cycles following a single GnRH injection and continuous infusion of GnRH were also noted with intermittent injections of GnRH in cows in the postpartum period.

The response of cows to different GnRH protocols has been variable because treatments were administered under different treatment conditions and times after parturition. In addition, the ovarian response has been variable. A single injection of GnRH induces ovulation in 43% of nursing beef cows 21 days after parturition (Fonseca et al., 1980). Similar studies in beef cows involving multiple injections of GnRH induce ovulation in 73 to 80% (Walters et al., 1982c) and 10 to 42% of cows (Edwards et al., 1983).

Another exogenous hormone, estradiol, has been used in the postpartum period to induce ovulation because of its ultimate positive feedback effect on LH and FSH release in cattle. Peters (1984b) showed that an estradiol implant in the early postpartum period could induce a surge in LH and FSH. An estradiol implant for 21 days in postpartum beef cows increased the proportion of cows that ovulated when this treatment was begun after day-25 postpartum.
(Garcia-Winder et al., 1988). It has been suggested that a norgestomet implant affects the selection and maturation of the dominant follicle, which results in the production of LH and estradiol (Garcia-Winder et al., 1986, 1987). Thus, exogenous progesterone during the postpartum period would allow the dominant follicle to undergo final maturation and ovulation, which would then, hopefully, be followed by a normal-length estrous cycle.

**Oocyte and Embryo Quality in the Postpartum Period**

In cattle, ovaries obtained from abattoirs constitute a continuous source of oocytes for research, although the quality of these oocytes is variable. The use of specific criteria in the selection of oocytes aids in the identification of the oocytes that could succeed in an IVF program (DeLoos et al., 1989). Most of the oocytes used for cattle IVF programs are aspirated from small antral follicles (2 to 7 mm in diameter). Smith et al. (1996) indicated that the developmental competence of oocytes from small antral follicles is not adversely affected by the presence of a dominant follicle, and this competence is also not dependent on either follicle size or stage of the estrous cycle when oocytes were harvested (Leibfried and First, 1979). Likewise, there was positive correlation between oocyte diameter and follicle size. As follicle size increased beyond 3 mm, the growth in oocyte diameter appears to plateau at ~120 µm (Fair et al., 1995). Bovine oocytes are most often obtained from aspirating small- and medium-size follicles (2 to 8 mm of diameter) (Bevers et al., 1997). However, blastocysts produced from bovine oocytes originating from >6 mm follicles have been reported to be double that of oocytes harvested from 2 to 5 mm follicles (Lonergan et al., 1994; Blondin and Sirard, 1995; Arlotto et al., 1996).

Antral follicles are characterized by the presence of a fluid filled cavity (the antrum). Follicles in the early antral stage reach a diameter of 180 to 250 µm in cows (Monniaux et al., 1984). Oocytes in antral follicles are still immature (meiotically arrested at the diplotene stage of the first meiotic division and the nucleus is enclosed in the germinal vesicle). In the cow, folliculogenesis requires up to 12 weeks from the primary to antral stage and ≥3 weeks are needed to complete folliculogenesis to a pre-ovulatory follicle stage (Ireland, 1987; Britt, 1991).

De Loos et al. (1989) have classified bovine oocytes into four categories based on compactness and transparency of the cumulus investment and homogeneity and transparency of the ooplasm as follows: Category 1 includes compact multi-layered cumulus investment, homogeneous ooplasm, total cumulus oocyte complexes (COC) light and transparent; Category 2 includes compact multi-layered cumulus investment, homogeneous ooplasm but with a coarse appearance and a darker zone at the periphery of the oocyte, total COC slightly darker and less transparent than Category 1; and Category 3, less compact cumulus investment, irregular
ooplasm with dark clusters, total COC is darker than Categories 1 and 2. Category 4 includes an expanded cumulus investment, cumulus cells scattered in dark clumps in a jelly matrix, irregular ooplasm with dark clusters with a total COC dark and irregular.

The quality of oocytes harvested during the postpartum period has not been widely studied. There are some factors such as the environment of the uterus, hormonal and environmental factors that impact the oocyte development and fertility after parturition. Caamaño (1999) has suggested that immature oocytes harvested from beef and dairy cows between day-30 to day-60 postpartum have the potential to be fertilized and to develop into embryos after being used in IVM, IVF and IVC procedures. The oocytes of excellent and good quality harvested from dairy cows did not differ from oocytes obtained from slaughterhouse ovaries.

Also, 4 beef cows were subjected weekly to transvaginal oocyte collections for 3 weeks starting 30 days postpartum (Caamaño, 1999). A total of 37 oocytes were recovered of which 37% of the recovered oocytes were classified as excellent and good quality. The cleavage rate was 20% and only one blastocyst was produced. In this study, likewise, administration of exogenous FSH to postpartum cows did not increase either the number of ovarian follicles or the quality of oocytes harvested from these postpartum cows.

Other Factors that Affect Fertility in the Postpartum Period

During the postpartum period cows undergo a transition from an anestrous state, which is characterized by infertility, into cycling animals with an opportunity for a pregnancy. However, there are various factors including the replenishment of LH stores in the anterior pituitary, short estrous cycles and uterine involution that could affect embryo development in the uterus during this transition. Researchers have been trying for years to establish pregnancies in the early postpartum period.

Oocyte quality has been related to fertility in the postpartum period. It has been established that short estrous cycles occur after the first ovulation in the postpartum cow. Premature secretion of PGF₂α is thought to be responsible for causing premature luteal regression in beef cows (Peters et al., 1984a; Copelin et al., 1987; Zollers et al., 1991). To overcome this physiological state, pre-treatment of cows with a progestin helps to form luteal tissue with a normal luteal function in response to weaning or gonadotropin treatments (Ramirez-Godinez et al., 1981). Progesterone treatment increases the number of progesterone receptors in the uterus at ~5 days post-estrus in cattle (Zollers et al., 1993). Thus, fertility may be improved by pre-treatment with progesterone in the postpartum period by preventing the typical shortened luteal phase of the estrous cycle (Butcher et al., 1992; Inskeep, 1995).
Breuel et al. (1993b) evaluated the factors that affect fertility in beef cows with short estrous cycles compared with normal-length estrous cycles. The factors related to fertility were the oocyte and the early uterine environment. In this research, cows were divided into two groups, those with normal-length estrous cycles and those with short estrous cycle. The oviducts were removed and embryos collected 3 days after mating. The oocyte released at the beginning of a short luteal cycle was capable of being fertilized, undergoing early development and transported into the uterus. However, when the cows were supplemented with exogenous progesterone, they failed to maintain the pregnancy in cows with a short estrous cycle. It was proposed that the low fertility in a short cycle was not only due to premature luteal regression but also could result from the ovulation of an oocyte with an inherent defect that prevents its developing in the oviduct or the uterus.

Short luteal cycles are not the only limiting factor in low pregnancy rates. Other factors may include quality of the oocytes or embryos and/or inadequate preparation of the uterus to support pregnancy. Butcher et al. (1992) studied the ability of the early postpartum uterus to support pregnancy with short or normal-length luteal phases when cows were administered exogenous progesterone. The uterus of some postpartum cows with short estrous cycles is capable of maintaining a pregnancy, if provided a good quality embryo and twice daily injections of progesterone (Butcher et al., 1992).

Schrick et al. (1993) compared embryos from cows with short estrous cycle with embryos from cows with normal-length estrous cycle in their ability to maintain pregnancy when they were transferred on day-6 into nonlactating cyclic recipients. The fertilization rate and quality or stage of development of day-6 embryos did not differ between cows with short and normal-length estrous cycles when embryos were transferred into the uterus of cycling cows. The pregnancy rate from oocytes recovered from short estrous cycle cows was only 13% compared with 32% for those recovered from normal-length estrous cycle cows. It was also concluded that PGF$_2$α in the uterine lumen might also contribute to the high embryonic mortality in exhibiting a short estrous cycle (Schrick et al., 1993).

These results suggest that the environment of the uterus is the factor responsible for the higher embryonic loss in cows with short estrous cycles when PGF$_2$α concentrations were correlated to fertility. Oocytes and embryos originating from cows with short estrous cycles did not maintain pregnancy rates. Poor pregnancy rates are likely be due not only to the environment of the uterus but also to the quality of the oocyte in the postpartum period.
Transvaginal Ultrasound-Guided Oocyte Aspiration

Diagnostic ultrasound has been used to study the internal reproductive tract in large domestic animals via the transrectal route since the early 1980s (Pierson et al., 1988). Real time ultrasonography has proved to be a rapid and reliable technique for studying reproductive functions in cattle. The main components of this common approach are the vaginal transducer (5 MHz) and the console (Rajamahendran et al., 1994).

There are different methods for recovering immature bovine oocytes for the production of embryos for IVF programs. Oocytes can be collected from abattoir ovaries; however, the lack of information on the donor genetics is considered the limitation for its commercial use. Another option is obtaining fresh oocytes from live donor cows for IVM, IVF and IVC. One of the first methods to obtain bovine oocytes was via laparoscopic oocyte collection (Holland et al., 1981; Lambert et al., 1983); however, this method is now considered to be too traumatic for in field use. Subsequently, a transvaginal ultrasound method for recovering fresh oocytes was reported in humans (Dellenbach et al., 1985).

Transvaginal ultrasound-guided oocyte aspiration (TUGA) was developed as a new assisted reproductive technology to provide oocytes for fertilization in research programs. This technology was first described in the University of Utrecht, Netherlands by Dr. Pieterse and his colleagues (Pieterse et al., 1988). This technology is now being considered as an alternative to traditional embryo transfer programs for the production of cattle embryos (Pieterse et al., 1991b; Kruip et al., 1991; Scott et al., 1994).

Transvaginal Ultrasound-Guided Oocyte Aspiration in the Early Postpartum Cow

One of the potential applications of real time ultrasonography in cattle reproductive management is in the postpartum cow. However, only a few ultrasound studies have been reported using this technology on postpartum females (Kähn, 1992; Rajamahendran et al., 1994). In these studies the main objective was to evaluate the progress and the completion of uterine involution. This ultrasound application (uterine evaluations) has been reported in the postpartum period in cows (Okano and Tomizuka, 1987), mares (Griffin and Ginter, 1991) and dogs (Yeager and Concannon, 1990).

In a preliminary study, TUGA has been applied in the late postpartum period (≥ 30 days) in beef and dairy cows (Caamaño, 1999). This research was conducted with the objective of evaluating the developmental competence of oocytes recovered from postpartum dairy and beef cows. In this preliminary study, Caamaño (1999), suggested that immature oocytes harvested from postpartum cows between day-30 to day-60 postpartum have the potential to be fertilized and possibly to develop into embryos.
CHAPTER II

TRANSVAGINAL ULTRASOUND-GUIDED OOCYTE ASPIRATION FROM FSH-TREATED POSTPARTUM BEEF COWS

Introduction

Real-time ultrasonography has been used as a research tool for the examination of reproductive organs in female farm animals. Although the first report of the application of ultrasonography for pregnancy detection in sheep was made over 35 years ago, the interest in the application of real-time ultrasonography started in the late 1970s.

The laparoscopic follicle oocyte aspiration technique was first described as a method of recovering human oocytes in 1970 (Steptoe and Edwards, 1970). In cows, oocyte aspiration using endoscopy via the paralumbar route started in the early 1980s (Lambert et al., 1983). This technology was adaptable for the study of the reproductive tract in large domestic animals via the transrectal route in a nonsurgical method for bovine oocyte recovery from live animals (Pierson et al., 1988; Pieterse et al., 1991b).

Transrectal ultrasonography and ultrasonography have been used as a diagnostic tool since the 1980s in large nondomestic species (Adams et al., 1991), cattle (Pierson and Ginther, 1984; Reeves et al., 1984; Edmondson et al., 1986b; Pierson and Ginther, 1988; Rajamahendran and Taylor, 1990; Beal et al., 1992; Rajamahendran et al., 1994), ewe (Bartlewski et al., 2000) and swine (Soede et al., 1992). The use of transvaginal ultrasound-guided oocyte aspiration has been reported in cattle (Pieterse et al., 1991b, 1992; Gibbons et al., 1994; Kruip et al., 1994; Scott et al., 1994; Bungartz et al., 1995; Garcia and Salaheddine, 1998), calves (Brogliatti and Adams, 1996; Fry et al., 1998), humans (Lenz et al., 1981; Wikland et al., 1983; Dellenbach et al., 1985; Yee, 1988), horses (Brück et al., 1992, 1997; Cook et al., 1992; Meintjes et al., 1995b), swine (Bellow et al., 2001) and endangered species (Pope et al., 1995; Asa et al., 1998).

Ultrasoundography also has been used to assess postpartum uterine involution in cows (Okano and Tomizuka, 1987) and dogs (Yeager and Concannon, 1990) and to evaluate pathological conditions of the postpartum period in cows (Bakana et al., 1994). The transvaginal ultrasound-guided oocyte aspiration technique has been also used widely to harvest bovine oocytes to be used in IVF programs (Pieterse et al., 1989, 1991b; Kruip et al., 1993; Rath, 1993; Kruip et al., 1994). It was originally established by Pieterse et al. (1989) and is the most commonly used method for transvaginal oocyte recovery in cattle (Kruip et al., 1990; Pieterse et al., 1991a; Looney et al., 1994; Bungartz et al., 1995; Fry et al., 1998). Transvaginal ultrasound-
guided oocyte aspiration (TUGA) can be conducted once or twice-a-week for several months and up to 3 months of pregnancy in cows (lactating and nonlactating) (Reinchenbach et al., 1993; Gibbons et al., 1994; Meintjes et al., 1995a; Garcia and Salaheddine, 1998) and buffalo cows (Boni et al., 1996). It is proposed that repeated recovery of oocytes can produce more embryos than might be possible by standard embryo transfer programs (Kruij et al., 1994).

Exogenous hormones such as FSH, equine chorionic gonadotropin (eCG), GnRH and bovine somatotropin (bST) have been used to improve the availability of follicles and oocyte recovery rates (Pieterse et al., 1992; Irvine et al., 1993; Ryan et al., 1993; Looney et al., 1994; Hwang et al., 1997; Riddell et al., 1997). Some of these protocols have been used in pregnant cows during the first trimester of gestation obtaining good recovery oocyte rates (Ryan et al., 1993; Meintjes et al., 1995a). The ultrasound guided-transvaginal oocyte aspiration technique has also been used to harvest oocytes from prepubertal calves (Brogliatti and Adams, 1996; Brogliatti et al., 1997).

This oocyte recovery technique combined with IVM, IVF and IVC is a method for producing embryos that are suitable to transfer and produce pregnancies (Looney et al., 1994). Oocytes derived from abattoir ovaries had similar survival rates under in vitro conditions as bovine oocytes collected by the ultrasound-guided method (Kruij et al., 1990; Gibbons et al., 1995).

The postpartum period has been the objective of numerous studies involving endocrine and physiological aspects because it plays an important role in the continuity of cattle operations. In these studies the main objective has been to improve the fertility and productivity of the postpartum cow. There are also attempts to induce superovulation with FSH in postpartum dairy cows (Kaminura et al., 1996) resulting in good production of follicles (>10 mm); however, embryos that are produced in cows before day-30 postpartum were reported to be of lower fertility (Schrick et al., 1993). Information concerning the quality of oocytes produced during the postpartum period is insufficient and may be affected by other factors, such as uterine status and environmental factors. We feel that ultrasound-guided transvaginal oocyte aspiration could be an alternative reproductive tool to produce oocytes in the postpartum period. Transvaginal oocyte aspiration could be used in IVM, IVF and IVC programs to produce embryos from postpartum dairy and beef cows after 30 days postpartum (Caamaño et al., 1999) and during the first 20-days postpartum (Perez et al., 2001a, 2001b). However, the potential for the production of oocytes, embryos, pregnancies and calves in early postpartum beef cows has not yet been investigated. The objective of this study was to evaluate the use of FSH for oocyte production in early postpartum beef cows.
Materials and Methods

Experimental Animals

Twenty multiparous crossbred nursing postpartum beef cows with a moderate body condition (between 5 to 7) (scores 1 through 9; Richards et al., 1986) were randomly assigned to one of two treatment groups (FSH and no FSH). The FSH treatment group (n=10) received a total of 32 mg dose of FSH (FSH-P, Sioux Biochemical. Sioux Center; IA, Lot No. 3090, with LH contamination of <6%) (im) in descending doses daily for a 4-day period. The FSH treatment started at day-21 postpartum for the first oocyte aspiration and day-31 for the second oocyte aspiration.

The control group (n=10) received a similar total volume (6.5 mL) of saline carrier solution (im) over a 4-day period. Prior to the beginning of the experiment, follicles (>5 mm in diameter) were aspirated (follicle reduction) at day-15 postpartum in both treatment groups. Oocyte collections using a standard transvaginal ultrasound-guided oocyte aspiration procedure (Meintjes et al., 1995a) were performed on day-25 and on day-35 postpartum (each ~18 hours after the last dose of FSH or saline vehicle) (Figure 2.1).

Experimental Design

Cows were randomly assigned to one of four combination treatment groups using a 2 x 2 factorial arrangement design with treatment (FSH and no FSH) and time of treatment postpartum (aspirations at day-25 and day-35 postpartum). Ten cows were allotted to each treatment and each time combination. These treatments were: FSH with oocyte aspiration at
day-25 postpartum (Treatment 1), saline carrier vehicle with no FSH with oocyte aspiration at day-25 postpartum (Treatment 2), FSH with oocyte aspiration at day-35 postpartum (Treatment 3) and carrier vehicle with no FSH with oocyte aspiration at day-35 postpartum (Treatment 4).

**Oocyte Recovery Procedure**

Cows were restrained in a stock chute and prepared for the transvaginal ultrasound-guided oocyte aspiration procedure. Each cow received 5 to 7 mL of lidocaine (Lidocaine Hydrochloride 2%, Butler, Columbus, OH) to induce an epidural anesthetic effect.

An Aloka 500-V real time ultrasound scanner (Corometrics Medical Systems, Inc. Wallingford, CT) was used with a 5 MHz array transducer, which was fixed at the tip of a plastic probe with a stainless steel needle guide. A 17-gauge, 50-cm stainless needle was used to aspirate the follicles. The 17-gauge needle was connected to 16-gauge polyethylene tubing and attached to an Em-Con® filter (Professional Embryo Transfer Supply, PETS, Canton, TX). The aspiration of follicles was performed with the aid of a regulated vacuum pump (Cook Veterinary Products, Australia) at 70 mm Hg of negative pressure. The vacuum pressure was adjusted between each aspiration. After the ovary was visualized, the oocyte aspiration needle (previously introduced into the needle guide) was inserted through the vaginal wall and into the ovarian follicles. Negative pressure was applied as the needle entered into each follicle to enable recovery of the oocyte.

All follicles >5 mm in diameter were aspirated. In this study, the collection medium consisted of Dulbecco’s phosphate-buffered saline (Gibco, Grand Island, NY) with 1% calf serum (Gibco-BRL, Life Technologies, Grand Island, NY), 5 units/mL of heparin (Elkins-Sinn, Inc., Cherry Hill, NJ) and 0.5 mg/mL of gentamicin (Gibco-BRL, Life Technologies, Grand Island, NY).

**Oocyte Evaluation**

After the oocyte aspiration, the Em-Con® filter was rinsed 2 or 3 times and then the aspirated medium was placed into a square 10 x 10 cm Petri dish (Becton-Dickinson Labware, Lincoln Park, NJ) and oocytes were located using a stereomicroscope. Once the oocytes were located, they were transferred to another Petri dish containing Tissue Culture Medium-199 (TCM-199; Gibco-BRL, Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, Hi Clone Laboratories, Logan, UT) and 1 mg/mL of gentamicin. The recovered oocytes were divided into two groups based on the presence of and number of cumulus cell layers surrounding the oocytes. Oocytes that had more than two complete layers of cumulus cells were classified as “excellent” or “good” (Category A) and the oocytes that had less than two complete layers of cumulus cells were placed in the “poor” classification category (Category B).
Oocyte Culture for In Vitro Maturation

After rinsing 2 times in TCM-199, oocytes were cultured for 24 hours to mature in vitro. The culture medium was conducted under mineral oil in 50 µL droplets of TCM-199 with 10% FBS, 5 µg/mL of FSH (Sigma Chemical Co. St. Louis, MO), 10 µg/mL of LH (Sigma Chemical Co. St. Louis, MO) and 10 µL of gentamicin at 39°C with an atmosphere of 5% CO₂ in humidified air.

In Vitro Fertilization

After a 24-hour maturation period, oocytes were removed from the maturation medium. Oocytes were washed twice, transferred and placed in 50 µL of Brackett-Oliphant medium (Brackett and Oliphant, 1975). Semen from a fertile Holstein bull (CSS 7H3429, INKA Rotate Adam-ET, No. 2044052) was thawed in a water bath at 36°C for 45 seconds and placed in Brackett-Oliphant medium. Semen was deposited into a 15-mL centrifuge tube with 6 mL of fertilization medium and centrifuged twice at 200 x g for 6 minutes. Sperm cells were counted by hemocytometer and then diluted with equilibrated fertilization medium. Oocytes were in vitro fertilized with 50 µL of semen with a concentration of 5 x 10⁶ sperm/mL and then incubated in 50 µL microdroplets (100 µL total volume) of IVF medium. The in vitro culture was performed at 39°C in a humidified incubator with a 5% CO₂ during a 16-hour interval.

In Vitro Culture

After 16 hours of incubation with sperm, oocytes were removed and washed twice with bovine embryo culture medium (BECM) with 4% bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO) and then transferred to 40-µL droplets under mineral oil in a 35 x 10 mm Petri dish (Becton-Dickinson Labware, Lincoln Park, NJ). The in vitro culture was conducted at 39°C in an atmosphere of 5% CO₂ in air. Embryo development (cleavage) was assessed at 48 hours after IVF. The cleaved embryos were cultured on a Buffalo Rat Liver (BRL) cell monolayer in BECM for 7 days or until they reached the expanded blastocyst stage.

Blood Sampling

Blood samples (8 mL) were collected from the jugular vein on day-15, day-25 and day-35 postpartum and centrifuged within 30 minutes after collection to separate the serum. Serum samples were frozen at -20°C and used for determination of progesterone (P₄) concentrations by radioimmunoanalysis (RIA; DSL-3400 Progesterone Kit, Diagnostic Systems Laboratories, Webster, TX).

Statistical Analysis

The mean number of follicles produced, oocyte recovery rate, oocyte quality and embryo development data were analyzed using a General Linear Model procedure (GLM) with the
model statement including treatment (FSH and no FSH), time (aspiration from day-25 and day-35 postpartum treatment groups) and any treatment interactions. Treatment, time and the interaction of these two terms were used as class variables in this model. A multiple comparison analysis of the treatment LS-means was performed with a Tukey’s adjustment test on the differences between LS-means of each treatment combination. Treatment and time were designated as fixed effects and experimental cows were used as in the random experimental error term. The resulting 2 x 2 factorial design consisted of four treatment combinations as follows: FSH with oocyte aspiration at day-25 postpartum (Treatment 1), saline vehicle with no FSH with oocyte aspiration at day-25 postpartum (Treatment 2), FSH with oocyte aspiration at day-35 postpartum (Treatment 3) and carrier vehicle with no FSH with oocyte aspiration at day-35 postpartum (Treatment 4). Ten cows were used for each treatment and time combination. A mixed-effect linear model was fit for the analysis of variance. The interaction of these two main effects was checked with strict additivity. All analysis was performed with the SAS® System for Microsoft Windows®, 1999-2001 by SAS Institute Inc., Cary NC, SAS proprietary Software Release 8.2 (TS2MO), Licensed to Louisiana State University.

Results

The average number (mean ± SE) of follicles (>5 mm in diameter) per cow on day-15 postpartum (follicle reduction) was 2.1 ± 0.4 and 1.9 ± 0.4 for the FSH and control treatment groups, respectively (Figure 2.2).

![Figure 2.2. Mean number of follicles (>5 mm in diameter) in beef cows at day-15 postpartum.](image-url)
Overall, the postpartum cows in this study had a positive response to the FSH treatment (Figure 2.3 and 2.4). The total number of follicles (>5 mm in diameter) per cow across both oocyte aspirations was 26.3 ± 4.3 follicles for the FSH-treated group and 4.8 ± 0.5 follicles for the control group. The total number of follicles (>5 mm) aspirated per cow from both oocyte collections was 23.4 for the FSH-treatment group and of these follicles, the number of oocytes recovered was 14.7 (63% recovery rate). In the control group, the total number of follicles aspirated per cow was 4.0, resulting in 2.6 oocytes recovered (65% recovery rate)(Figure 2.3). The total number of follicles present on the ovaries, the total number of follicles aspirated and the total number of oocytes recovered per cow in both collections in the FSH-treated groups were significantly greater than those in the saline-treated control group. The average oocyte recovery rate; however, was not significantly different between the FSH and control treatment groups at 63% and 65%, respectively.

![Figure 2.3. Total overall oocytes produced per female from FSH-treated and control postpartum beef cows.](image)

Correspondingly, the total number of follicles (>5 mm in diameter) per cow at day-25 postpartum was 14.6 ± 2.3 in the FSH treatment group. Also, the number of follicles aspirated per donor was 13.2 ± 2.4, resulting in 8.6 ± 1.3 oocytes aspirated (65% recovery rate). In the control group the total number of follicles (>5 mm) per cow at day-25 postpartum was 2.5 ± 0.3. The day-25 FSH-treated group was significantly different than the control group, where only 2.0
Figure 2.4. Ovarian response to FSH treatment in postpartum beef cows.
Table 2.1. Mean (± SE) oocytes produced per female from FSH-treated and control beef cows during the first oocyte aspiration at day-25 postpartum

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of follicles</th>
<th>No. of follicles aspirated</th>
<th>No. of oocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td>14.6 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.2 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.6 ± 1.3&lt;sup&gt;a&lt;/sup&gt; (65%)</td>
</tr>
<tr>
<td>No FSH</td>
<td>2.5 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3 ± 0.4&lt;sup&gt;b&lt;/sup&gt; (65%)</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means within columns with different superscripts are significantly different (P<0.05).

Table 2.2. Mean (± SE) oocytes produced per female from FSH-treated and control beef cows during the second oocyte aspiration at day-35 postpartum

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of follicles</th>
<th>No. of follicles aspirated</th>
<th>No. of oocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td>11.7 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.2 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.1 ± 1.3&lt;sup&gt;a&lt;/sup&gt; (60%)</td>
</tr>
<tr>
<td>No FSH</td>
<td>2.0 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3 ± 0.4&lt;sup&gt;b&lt;/sup&gt; (65%)</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means within columns with different superscripts are significantly different (P<0.05).
± 0.3 follicles were aspirated and 1.3 ± 0.4 oocytes were recovered (65% recovery rate) (Table 2.1).

The total number of follicles per cow and the number of follicles aspirated from donors in the day-35 FSH-treated group was also greater than the control group. In the day-35 FSH-treated group, 11.7 ± 2.1 follicles per cow were produced, of which 10.2 ± 2.4 follicles were aspirated, resulting in 6.1 ± 1.3 oocytes recovered (60% recovery rate). In the control group 2.0 ± 0.3 follicles were found per cow, of which 2.0 ± 0.4 follicles were aspirated, resulting in 1.3 ± 0.4 oocytes recovered (65% recovery rate) (Table 2.2). The average oocyte recovery rate was not significantly different between the FSH and control treatment groups at 60% and 65%, respectively.

The mean number of oocytes (excellent and good category) per cow in both oocyte collections was significantly different with 13.7 and 2.1 for the FSH-treated and control groups, respectively (Tabular data not presented). In the FSH-treated group the excellent and good quality oocyte number were lower for cows on day-25 postpartum than for cows on day-35 postpartum with 7.2 and 6.5 excellent and good oocytes per donor, respectively. The quality score improved for oocytes from the day-25 postpartum (1st oocyte aspiration) to the day-35 postpartum (2nd oocyte aspiration) in the control treatment with scores of 0.6 and 1.5 oocytes per cow, respectively. As expected, the FSH-treated group had greater oocyte production (7.2 and 6.5 excellent and good quality oocytes per donor) than the control groups (1.3 and 1.3 oocytes) on day-25 and day-35 postpartum, respectively. The number of poor quality oocytes per donor was not statistically different among treatments in this study.

The number of blastocysts produced per cow was 1.1 ± 0.3 and 0.2 ± 0.1 for the FSH-treated group and the control group, respectively. The in vitro embryo production from cleaved embryos was greater from the FSH-treated group compared with the control group. When oocytes collected from the FSH-treated cows (14.7 ± 2.4) were compared with oocytes recovered from control cows (2.6 ± 0.6), significantly cleavage and blastocyst rates (P<0.05) resulted from oocytes harvested from FSH-treated cows.

Circulating progesterone levels at day-15, day-25 and day-35 postpartum were: 1 ± 0.6, 0.5 ± 0.2 and 1.4 ± 0.9 ng/mL for the FSH-treated group and 0.5 ± 0.2, 0.4 ± 0.2 and 1 ± 0.6 ng/mL for the control group, respectively (Figure 2.5). In this study, there was no evidence to indicate that functional luteal tissue formed after the oocytes were mechanically removed from the follicles on day-25 or day-35 postpartum.
The use of reproductive technologies such as transvaginal ultrasound-guided oocyte aspiration can be applied to early postpartum beef cows to harvest oocytes that can be used in IVF programs. An early effort was made in a preliminary study to attempt this technique in beef and dairy cows after 30 days postpartum (Caamaño, 1999).

In the present study, the first transvaginal ultrasound guided oocyte aspiration was performed at day-25 postpartum. This was executed during uterine involution, which is most often completed at day-35 to day-40 postpartum in most mature beef cattle (Gier and Marion, 1968; Spicer et al., 1986b; Okano and Tomizuka, 1987).

Even though uterine involution was in progress at day-25 postpartum, manual manipulation of the reproductive tract to aspirate oocytes from both ovaries was performed with little difficulty. Ultrasonography of the ovaries of beef cows at day-15 after parturition in the present study showed the presence of follicles >5 mm in diameter. The number of follicles >5 mm was 2.1 and 1.9 for the FSH-treated and control group, respectively. In suckled beef cows, medium-sized and dominant follicles are often present by day-10 to day-20 postpartum (Spicer et al., 1986b; Breuel et al., 1993a; Crowe et al., 1993). Ultrasound images at day-15, day-25 and day-35 postpartum show the presence of follicles.

Figure 2.5. Circulating progesterone levels (ng/mL) in postpartum beef cows at day-15, day-25 and day-35 postpartum.

Discussion

The use of reproductive technologies such as transvaginal ultrasound-guided oocyte aspiration can be applied to early postpartum beef cows to harvest oocytes that can be used in IVF programs. An early effort was made in a preliminary study to attempt this technique in beef and dairy cows after 30 days postpartum (Caamaño, 1999).

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Figure 2.5. Circulating progesterone levels (ng/mL) in postpartum beef cows at day-15, day-25 and day-35 postpartum.
postpartum and circulating progesterone levels showed the absence of a CL in all experimental cows in this study. Moller (1970) and LaVoie et al. (1981) also showed the absence of CL in beef cows during this same segment of the postpartum period. In the present study, there was no evidence to indicate that functional luteal tissue formed after the oocytes were mechanically removed from the follicles between day-25 and day-35 postpartum.

The aspiration of follicles using the transvaginal ultrasound-guided oocyte aspiration technique has been used widely in different physiological stages in dairy and beef cows. In a preliminary study, Caamaño (1999) used the transvaginal ultrasound-guided oocyte aspiration in postpartum dairy and suckled beef cows 30 days postpartum but little or no information was given on IVF-derived embryos or transfer results.

The characterization of follicular growth by waves (Sirois and Fortune, 1988) in prepubertal, cycling, pregnant and postpartum cows (Kastelic, 1994), allows the use of FSH to increase the growth of the follicles. Furthermore, FSH protocols have been used in cyclic cattle with transvaginal ultrasound-guided oocyte aspiration studies (Gibbons et al., 1994; Looney et al. 1994; Bungartz et al., 1995; Bordignon et al., 1997).

The results of the present experiment indicate that ovaries from early postpartum beef cows are capable of responding to a FSH treatment at day-21 and again at day-31 postpartum. Oocytes that were recovered using the transvaginal ultrasound-guided oocyte aspiration procedure are also capable of undergoing IVF and development to a blastocyst stage embryo. In this study, the ovaries from postpartum beef cows had good responses to the FSH treatment. The follicle response in FSH-treated postpartum beef cows was 26.3 follicles produced per cow. In contrast, the control group produced a significantly lower response with 4.8 follicles (>5 mm in diameter) for the two vehicle treatments also starting at day-21 and day-31 postpartum. Similar results have been reported in FSH-treated dairy cows at ~50 days postpartum, where the number of developing follicles >10 mm were increased when compared with the control treatment (Kamimura et al., 1996).

In contrast, Caamaño (1999) reported the presence of 17.1 small-size follicles, 2.3 medium-size follicles and 2.1 large-size follicles per cow during three oocyte aspirations with no hormonal stimulation in beef cows at day-30 postpartum. Exogenous FSH has been shown to increase follicle production in postpartum cows (Kamimura et al., 1996), problem cows (Looney et al., 1994), cycling dairy cows (Bungartz et al., 1995), prepubertal calves (Brogliatti et al., 1995) and pregnant cows (Meintjes et al., 1995a); however, FSH-treated dairy cows were found not increase the follicle production at the time of oocyte aspiration starting at day-30 postpartum (Caamaño, 1999).
In the present study, the follicle production from the first FSH stimulation protocol was 14.6 follicles per cow collected at day-25 postpartum; however, this level of follicle production tended to decrease by the time of the second hormonal stimulation to 11.7 follicles per cow collected at day-35 postpartum. These results could be due to the decrease in the recruitment from the cohort of follicles in the ovaries (Thatcher et al., 1996) because the two FSH stimulation protocols were performed during a 10-day interval. In contrast, the response was constant for the first oocyte aspiration with 2.5 follicles per cow compared with the second oocyte aspiration with 2.5 follicles in the control group.

The overall recovery rate was 63% and 65% for the FSH-treated and control group, respectively. The recovery rates from the present study are within the range of those reported where TUGA has been used in beef cows varying from 24 to 75% (Pieterse et al., 1989; Looney et al., 1994; Bungartz et al., 1995; Bordignon et al., 1997).

An average of 14.7 oocytes were retrieved overall from each of the 10 FSH-treated postpartum beef cows and 2.6 oocytes for the control group in a 10-day period under two FSH treatments starting at day-21 and day-31 postpartum. The overall oocyte recovery for the FSH-treated group was 8.6 and 6.1 oocytes at the first and second oocyte aspiration, respectively. These results are comparable with the average of 6.3 oocytes harvested from problem donor cows (Looney et al., 1994), 7 oocytes in dairy cows (Bungartz et al., 1995), 6.1 oocytes in infertile cows (Riddel et al., 1997), 7.2 in prepubertal calves (Brogliatti et al., 1996), 7.2 oocytes in lactating cows (Bungartz et al., 1995) and 6.2 oocytes in beef cows (Gibbons et al., 1994).

The mean number of oocytes (excellent and good category) in the control treatment improved from the first oocyte aspiration to the second aspiration. In contrast, the mean number of oocytes (excellent and good category) was not improved between day-25 and day-35 postpartum in the FSH-treated group. The overall oocyte quality tended to increase as the postpartum interval increased in control cows.

The overall cleavage rate obtained was 50% for the FSH-treated and control treatments. In this study, these results are generally lower than those found in commercial IVF programs (Looney et al., 1994). In contrast, Bungartz et al. (1995), Caamaño (1999) and Meintjes et al. (1995a) obtained 60.4%, 75.4% and 67% cleavage rates, respectively. The embryo production rate from cleaved embryos was 14.8% and 15% for the FSH-treated and control cows. This lower cleavage and blastocyst rates in the present experiment could be the result of technician experience and the IVF protocol used.

The use of reproductive technologies, such as transvaginal ultrasound-guided oocyte aspiration can be applied to noncycling postpartum beef cows starting 3 to 4 weeks after calving.
without causing a change in nursing patterns of the calves or causing injury to the dam. This approach could be used to produce supplemental IVF embryo transfer pregnancies from valuable seedstock cows before the female begins to cycle naturally for mating or artificial insemination. In this study, an average of 14 to 15 additional oocytes was harvested per FSH-treated cow for IVF procedures before day-36 postpartum.

In summary, FSH-treated postpartum beef cows can be a source of oocytes for IVF programs by using transvaginal ultrasound-guided oocyte aspiration. Furthermore, this may be a practical alternative for embryo production from postpartum beef cows.
CHAPTER III
OOCYTE AND EMBRYO PRODUCTION FROM FSH-TREATED POSTPARTUM BEEF COWS SHORTLY AFTER CALVING

Introduction

Ultrasonography has been used to improve the reproductive management of cattle (Boyd and Omran, 1991; Beal et al., 1992). Ultrasonography has been used to evaluate the progress and the completion of uterine involution in cows (Okano and Tomizuka, 1987), camels (Sumant and Sahani, 2000) and dogs (Yeager and Concannon, 1990).

Using ultrasonography for aspiration of oocytes has applications for in vitro fertilization programs. The first methodology describing procedures to obtain oocytes from live animals caused trauma because laparoscopy and laparotomy procedures were used. The ultrasound-guided transvaginal oocyte aspiration (TUGA) technique in cattle was developed as a non-invasive method to obtain oocytes (Kruip et al., 1994; Scott et al., 1994; Garcia and Salaheddine, 1998). This transvaginal oocyte aspiration technique was first described in cows in the late 1980s (Pieterse et al., 1989). This technique has been proven to be a reliable procedure for obtaining oocytes from living cows (Callessen et al., 1987; Pieterse et al., 1990; Kruip et al., 1994; Looney et al., 1994).

Ultrasound-guided technology was initially applied in humans (Lenz et al., 1981; Dellenbach et al., 1985) and then in cattle (Pieterse et al., 1989, 1991b, 1992; Walton et al., 1993; Looney et al, 1994; Hill et al., 1998), horses (Brück et al., 1992; Cook et al., 1992; Brück et al., 1992), prepubertal calves (Duby et al., 1996), goats (Graff et al., 1999), pigs (Bellow et al., 2001), buffalo cows (Boni et al., 1996) and endangered species (Adams et al., 1991; Pope et al., 1995; Asa et al., 1998). Transvaginal ultrasound-guided follicular aspiration of oocytes from live cows combined with IVM, IVF and IVC can be an alternative procedure to produce embryos. This allows for a source of oocytes that could be collected from pregnant cows (Meintjes et al., 1995a), cycling cows (Kruip et al., 1991) and problem cow donors (Looney et al., 1994). Oocyte aspirations can be performed once-a-week or twice-a-week for up to 3 months in cows (Gibbons et al., 1994). Oocytes can also be collected from a donor at the beginning of the estrous cycle (Paul et al., 1995) corresponding to the emergence of the first follicular wave.

No detrimental effects of follicular aspiration have been observed in pregnant cows (Meintjes et al., 1995a) or in nonpregnant cows aspirated twice-a-week for extended periods (Kruip et al., 1994). This technique combined with in vitro embryo production is emerging as a successful method for obtaining good quality embryos (Gibbons et al., 1994).
recovered by TUGA have resulted in embryos from cyclic cows that are of sufficient quality to establish and maintain a pregnancy when transferred to recipients (Gibbons et al., 1995).

Exogenous hormones have been used to enhance follicular development for oocyte collection in cows (Pieterse et al., 1992; Looney et al., 1994; Riddell et al., 1997), heifers (Ryan et al., 1993) and calves (Irvine et al., 1993; Hwang et al., 1997). Some of these protocols have been used in Holstein and Jersey cows starting after 50 days postpartum to induce ovarian stimulation (Kamimura et al., 1996). It has been reported; however, that embryos produced in cows before 30 days postpartum were lower in viability (Schrick et al., 1993).

Embryos that are produced during the postpartum period can be affected by factors such as uterine environment and other environmental factors (Breuel et al., 1993b; Schrick et al., 1993). The TUGA technique could be an alternative method to obtain oocytes during this postpartum period because the oocytes are obtained from the ovaries and not destined to enter the uterus as embryos. In a preliminary study, Caamaño (1999) reported that transvaginal ultrasound-guided oocyte aspiration could be used to harvest oocytes from postpartum dairy and beef cows after day-30 postpartum. These oocytes were exposed to IVM, IVF and IVC in a conventional IVF program and the resulting embryos only had limited ability to develop.

It is proposed that this approach may provide an alternative reproductive method as an efficient source of oocytes for the production of IVF embryos before day-30 postpartum. Perez et al. (2000) demonstrated that FSH-treated beef cows at day-25 after parturition could be a source of good quality oocytes and embryos in the early postpartum period. The objectives of this study were to evaluate the follicular development response and oocyte quality of beef cows treated with FSH shortly after parturition and to assess the feasibility of using the TUGA technique for postpartum beef cows on day-5, day-10, day-15 and day-20 postpartum.

Materials and Methods

Experimental Animals

Forty multiparous crossbred nursing postpartum beef cows in moderate to good body condition with scores ranging from 5 to 7 (Richards et al., 1986) were used in this experiment. Cows in the FSH treatment group (n=20) received a 32 mg dose (sc) of FSH (Follicle Stimulating Hormone, Lot No. 3091, with <6% LH content; Sioux Biochemical, Sioux Center, IA) in descending doses over a 4-day period. The control group (n=20) treatment received a dose of 6.5 mL of a saline vehicle (sc) during a similar 4-day period.

Oocyte collections using a transvaginal ultrasound-guided oocyte aspiration procedure (Meintjes et al., 1995a) were performed on day-5, day-10, day-15 and day-20 postpartum (each
~24 hours after the last dose of FSH or vehicle). Control cows were monitored by using ultrasonography at 3-day intervals from day-5 to day-20 postpartum.

**Experimental Design**

Cows were randomly assigned using a 2 x 4 factorial arrangement to either a FSH treatment (FSH) or control (no FSH) treatment starting on day 1, 6, 11, 16 days postpartum and oocytes harvested on days 5, 10, 15 and 20 postpartum (T1, T2, T3 and T4) (Figure 3.1).

**Figure 3.1. Donor cow treatment and oocyte collection schedule.**

**Oocyte Recovery and Embryo Production Procedures**

To restrict peristaltic movement each cow was placed in a holding chute and received 5 to 7 mL of lidocaine (Lidocaine Hydrochloride 2%, Butler, Columbus, OH) for an epidural anesthetic effect prior to oocyte recovery. An Aloka 500-V real time ultrasound scanner (Corometrics Medical Systems, Wallingford, CT) was used with a 5 MHz array transducer. A 17-gauge, 50-cm stainless needle (Cook Veterinary Products, V-BOAS, Spencer, IN) was used to aspirate the follicles.
The aspiration of follicles was performed with the aid of a regulated vacuum pump (Cook Veterinary Products, Australia) at 65 mm Hg of negative pressure. In this study, only ovarian follicles >5 mm were aspirated from each female. The collection medium consisted of Dulbecco’s phosphate-buffered saline (Gibco, Grand Island, NY) with 1% calf serum (Gibco-BRL, Life Technologies, Grand Island, NY), 5 units/mL of heparin (Elkins-Sinn, Cherry Hill, NJ) and 0.5 mg/mL of gentamicin (Gibco-BRL, Life Technologies, Grand Island, NY).

The recovered cumulus oocyte complexes harvested were evaluated morphologically and then divided into four groups according to the presence of cumulus cell layers surrounding the oocytes: 1) oocytes that had more than four layers of compact cumulus cells were classified as “A”. Oocytes with 2 to 4 layers of cumulus cells were classified as “B”. Oocytes with a single layer of cumulus cell or denuded were classified as “C” and oocytes with expanded cumulus cells were classified as “D”. Oocytes with classification A and B were matured for 24 hours using TCM-199 containing 10% fetal bovine serum (FBS), 5 µg/mL of FSH (Sigma Chemical), 10 µg/mL of LH (Sigma Chemical) and gentamicin under mineral oil in a humidified atmosphere of 5% CO₂ at 39°C.

Following a 24-hour maturation period, oocytes were fertilized with frozen-thawed semen from one bull (Holstein CSS 7H3429, INKA Rotate Adam-ET 2044052). This semen was first treated with Brackett-Oliphant medium (Brackett and Oliphant, 1975) supplemented with BSA (0.3%, Sigma, A-7511). Final sperm concentration in the IVF medium was 1 x 10⁶ sperm/mL. Oocytes were incubated with sperm for 5 hours at 39°C in a humidified incubator with 5% CO₂.

Granulosa cells surrounding the zygotes were not removed after fertilization and formed a confluent monolayer during the *in vitro* culture (Fukui and Sakuma, 1980). The *in vitro* culture was conducted in CR1aa medium (Rosenkrans and First, 1994) at 39°C in an atmosphere of 5% of CO₂ in air for up to 8 days. Embryo development (cleavage) was verified at 48 hours after IVF to determine the cleavage rate and again at day-7 and day-8 post-insemination to obtain the blastocyst development rate.

**Statistical Analysis**

A general linear model was used to assess whether injection of FSH altered the number of follicles produced per animal in treatment groups in postpartum beef cows. Comparisons were made at day-5, day-10, day-15 and day-20 postpartum in cows receiving either FSH or saline treatment. In addition, at each time interval the following variables were also measured in cows receiving the FSH treatment: number of follicles aspirated per cow, number of oocytes per cow, number of oocytes that cleaved per cow and number of blastocysts produced by cow. A 2 x 4 factorial arrangement was used that included treatments (n=2) and collection days (n=4).
Treatment combination means were analyzed for differences and a P-value of less than 0.05 was considered a significant level in this study.

All statistical analysis were performed with the SAS® System for Microsoft Windows®, 1999-2001 by SAS Institute Inc., (Cary NC), SAS proprietary Software Release 8.2 (TS2MO), Licensed to Louisiana State University at site No. 0009396001.

Results

The mean number (± SE) of follicles (>5 mm in diameter) per female detected by ultrasonography at day-5, day-10, day-15 and day-20 postpartum in the FSH-treated group was: 20.0 ± 4.0, 19.6 ± 2.9, 20.2 ± 5.6 and 17.6 ± 4.7, respectively (Table 3.1). The control group had fewer follicles (>5 mm) per female (0.4 ± 0.2, 1.2 ± 0.4, 1.2 ± 0.4 and 1.4 ± 0.6) at day-5, day-10, day-15 and day-20 postpartum than for the same postpartum days in the FSH-treated group (P<0.001) (Figure 3.2).

Table 3.1. The number of follicles, oocytes and embryos produced per cow from FSH- and no FSH-treated postpartum beef cows

<table>
<thead>
<tr>
<th>Oocyte collection days</th>
<th>No of follicles* FSH</th>
<th>No FSH</th>
<th>No. of follicles* aspirated</th>
<th>Cleaved* oocytes</th>
<th>Blastocysts* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day-5</td>
<td>20.0 ± 4.0a</td>
<td>0.4 ± 0.2b</td>
<td>12.8 ± 2.6</td>
<td>7.6 ± 1.9 (59)</td>
<td>3.8 ± 3.4</td>
</tr>
<tr>
<td>Day-10</td>
<td>19.6 ± 2.9a</td>
<td>1.2 ± 0.4b</td>
<td>16.4 ± 2.5</td>
<td>10.2 ± 1.7 (62)</td>
<td>5.4 ± 3.5</td>
</tr>
<tr>
<td>Day-15</td>
<td>20.2 ± 5.6a</td>
<td>1.2 ± 0.4b</td>
<td>12.6 ± 2.7</td>
<td>10.0 ± 1.3 (79)</td>
<td>5.0 ± 3.6</td>
</tr>
<tr>
<td>Day-20</td>
<td>17.6 ± 4.7a</td>
<td>1.4 ± 0.6b</td>
<td>12.6 ± 4.0</td>
<td>7.4 ± 2.1 (59)</td>
<td>2.8 ± 1.4</td>
</tr>
</tbody>
</table>

* Per FSH-treated donor cows.

a,b Means in rows across treatment groups are significantly different (P<0.001).

A CL was not detected by ultrasonography in the control cows during the first 20 days postpartum. Follicle development; however, was detected in control cows starting after day-5 postpartum. The number of follicles (>5 mm in diameter) per cow at day-5 postpartum (0.4) was found to increase to 1.4 follicles per cow at day-20 postpartum (Figure 3.3).
Figure 3.2. Follicular development in FSH-treated beef cows during the first 20-days postpartum.

Figure 3.3. Follicle development in nontreated postpartum (PP) beef cows during the first 20-days postpartum.
All the cows responded to the FSH treatment during the first 20 days postpartum (Figure 3.4). The mean number of follicles (>5 mm) per female detected at day-5 postpartum was: 10.6 ± 1.6 and 9.4 ± 2.1 for the right and left ovary, respectively.

The lack of uterine involution in these females occasionally made it difficult to manually manipulate the ovary for ultrasonography and aspirate follicles at day-5 postpartum. The aspiration procedure, however, was most often completed in 15 minutes. The number of follicles aspirated (>5 mm in diameter) per donor for FSH-treated females at oocyte collection days of day-5, day-10, day-15 and day-20 postpartum was: 12.8 ± 2.6, 16.4 ± 2.5, 12.6 ± 2.7 and 12.6 ± 4.0, respectively (Figure 3.5). Of these follicles, the number of oocytes and percent recovered per cow was: 7.6 ± 1.9 (59% recovery rate), 10.2 ± 1.7 (62% recovery rate), 10.0 ± 1.3 (79% recovery rate) and 7.4 ± 2.1 (59% recovery rate) at day-5, day-10, day-15 and day-20 postpartum, respectively (Figure 3.6).

Overall, the number of follicles (>5 mm), number of follicles aspirated, oocytes recovered, percent recovery rate, number of oocytes that cleaved, number of blastocysts from cleaved embryos and percent blastocysts produced per FSH-treated donor was: 19.4, 13.6, 8.8, 64.8%, 4.2, 1.4 and 32.3%, respectively. Overall, when beef cows were administered FSH during the
Figure 3.5. Number of follicles aspirated and oocytes recovered per FSH-treated cow at day-5, day-10, day-15 and day-20 postpartum.

Figure 3.6. Recovery rate of bovine oocytes harvested from FSH-treated females on day-5, day-10, day-15 and day-20 postpartum.
first 20 days postpartum, a mean of 19.4 follicles per female were produced compared with only 1.1 follicles for the control group.

The number of good quality oocytes (oocyte quality classifications of A and B) in FSH-treated donor females was: 3.0 ± 1.5, 6.8 ± 1.9, 2.4 ± 1.2 and 3.4 ± 0.9 at day-5, day-10, day-15 and day-20 postpartum, respectively. The number of good quality oocytes from cows at day-10 postpartum was significantly (P< 0.05) greater than the number of good quality oocytes obtained from females at day-15 postpartum. Although the FSH-treated cows at day-15 postpartum had the highest number of (7.6 ± 1.4) for poor quality oocytes, this treatment had the greatest number of blastocysts produced per female (1.8 ± 0.7). The number and percent of blastocysts was: 1.4 ± 1.2 (37%), 1.6 ± 0.7 (30%), 1.8 ± 0.7 (33%) and 0.8 ± 0.2 (29%) for FSH-treated donor females on day-5, day-10, day-15 and day-20 postpartum, respectively.

Individual blastocysts were nonsurgically transferred to 9 available beef females (on day-7 or day-8 of the estrous cycle), of which 4 of 4 resulting in pregnancies (at day-40 of gestation) were obtained from the day-5 postpartum collection group. From the day-10 postpartum group, 2 of 3 recipient cows resulted in pregnancies and 1 of 2 resulted in pregnancy from the day-15 postpartum group. The first calf born of these treatments came from a day-10 FSH-treated beef cow. This healthy calf was a male and weighed 60.5 kg with a gestation length of 279 days (Figure 3.7).

**Discussion**

The use of the TUGA procedure has not been widely used in postpartum beef cows. Ultrasonography has been used in studies to evaluate the uterine involution in the postpartum cow (Okano and Tomizuka, 1987; Rajamahendran and Taylor, 1990; Rajamahendran *et al.* 1994). Also, TUGA has been used to study different reproductive physiological events in the postpartum period of the camel (Rajamahendran *et al.*, 1994; Sumant and Sahani, 2000). This technology provides a rapid noninvasive form of visual access to the internal reproductive organs (Pierson *et al.*, 1988; Pierson and Ginther, 1988).

This reproductive tool was used in the present study to monitor the stimulated ovaries and retrieval oocytes of FSH-treated postpartum beef cows. There is little information available relating to the use of TUGA procedure in the early postpartum period, specifically during the first days after parturition. In the present study, oocyte aspirations were performed from day-5 to day-20 postpartum in beef cows. Most of the studies using this technique have been applied to recover oocytes from cows in other different reproductive physiological stages (Hill *et al.*, 1998). TUGA has been performed in the postpartum period in dairy and beef cows after 30 days.
Figure 3.7. First IVF-derived calf born from a day-10 postpartum FSH-treated oocyte donor beef cow. This calf was a male and weighed 60.5 kg. The gestation length was of 279 days.
postpartum, in a preliminary study (Caamaño, 1999) and in FSH-treated beef cows at day-25 and day-35 postpartum (Perez et al., 2000).

This laboratory reported a production of 14.6 follicles per female in FSH-treated beef cows at 25 days after calving (Perez et al., 2000). Caamaño (1999) noted positive results (Overall of 21.5 follicles per cow) in postpartum nonstimulated beef cows during 3 weeks of oocyte aspiration starting at day-30 after parturition. In the present study, all the cows responded to the FSH-treatment during the first 20 days after parturition (19.4 follicles per cow). This response was similar in all of the FSH-treated groups at day-5, day-10, day-15 and day-20 after calving, with 20, 20, 20 and 18 follicles per cow, respectively.

The lower ovarian response to FSH in our study could be due to the recruitment and stimulation of follicles present in the early postpartum period by the FSH treatment. The follicular development during the early postpartum period has been characterized by growing follicles (Wagner and Hansel, 1969; Stevenson and Britt, 1979; Spicer et al., 1986b; Savio et al., 1990). Duffor and Roy (1985) reported the presence of 8 mm follicles on the ovaries of dairy cattle between day-15 and day-35, while Spicer et al. (1986b) reported the presence of 8 mm follicles by day-7 postpartum with an increase in their number between day-7 and day-42 in lactating beef cows.

Ultrasound examination during the early postpartum period (day-5 to day-15) in mature beef cows is often difficult to perform due to the enlarged uterus in dairy cows (Morrow et al., 1966). The follicle aspiration of females at day-5, day-10 and day-15 postpartum in FSH-treated cows; however, was successfully completed in the present experiment. In the 5-day postpartum FSH-treated group, it was occasionally difficult to manipulate the ovary ipsilateral to the previously pregnant horn, but in general the procedure was completed in 20 minutes.

Several studies have indicated that the postpartum period could be a source of oocytes in beef cattle (Caamaño, 1999, Perez et al., 2000, 2001a). In the present study, the number of follicles aspirated, oocytes recovered and percent recovery rate per cow at day-20 postpartum was 13.6, 8.8 and 64.8%, respectively. Cows treated with FSH immediately after parturition can be aspirated as early as 5 days postpartum. The oocytes recovered and percent oocyte recovery (13 follicles aspirated and 8 oocytes recovered, with a 59% oocyte recovery) was considered acceptable while developing the procedure.

After day-30 postpartum, Caamaño (1999) obtained 3 oocytes per beef cow and 49% oocyte recovery during 3 oocyte aspirations, with no hormonal stimulation. In this laboratory,
Perez et al. (2000) aspirated 13.2 follicles of which 8.6 oocytes were recovered with a 65% oocyte recovery in FSH-treated postpartum beef cows at day-25 postpartum. These results in the postpartum period were similar to those where oocytes were collected from pregnant cows with 11 oocytes per cow and 43% oocyte recovery rate (Meintjes et al., 1995a), cycling cows with 6.2 oocytes (Gibbons et al., 1994) and problem breeder cows with 8.6 oocytes with a 70% oocyte recovery rate (Looney et al., 1994).

Early FSH-treated postpartum beef cows were found to be a source of good quality oocytes. In this study, 44% of the oocytes collected during the first 20 days after calving exhibited excellent and good quality morphology. Caamaño (1999) found that 37% of the oocytes recovered after day-30 postpartum in nonstimulated beef cows were classified as excellent and good oocytes. Our study shows the potential role that good quality oocytes could have after parturition during the acyclic period in the postpartum cow. These oocytes can be fertilized and developed into embryos using conventional in vitro embryo production methods. In relation to blastocyst production, 32% of the excellent and good oocytes developed into blastocysts during the first 20 days after parturition. These results were unlike those reported by Caamaño (1999) where only 15% of the oocytes developed to the blastocyst stage.

The difference in blastocyst production could be due to the in vitro fertilization method used in these experiments. There is evidence that the oocytes produced during the postpartum period may not be the major problem in the postpartum cow as previously reported (Schrick et al., 1993; Inskeep, 1995). Since embryos were produced during the early postpartum, the ovaries apparently do not contribute to the low fertility of the postpartum period. In this study, selected blastocysts were nonsurgically transferred to available beef females (n=9), of which a 77% pregnancy rate (at day-40 of gestation) was obtained.

In summary, these results demonstrate that good quality oocytes produced from FSH-treated beef cows during the first 20-days postpartum can result in blastocyst production.
CHAPTER IV

TRANSVAGINAL ULTRASOUND-GUIDED OOCYTE ASPIRATION FROM FSH-TREATED POSTPARTUM DAIRY COWS

Introduction

Ultrasonography has been widely used as a tool to assess reproductive status in cattle (Edmondson et al., 1986; Fissore et al., 1986; Boyd and Omran, 1991; Beal et al., 1992). This technology has been adapted to study the internal reproductive organs using the transrectal route in large domestic animals. One of the applications of using ultrasonography is during the postpartum period, where this approach has been used primarily to evaluate the uterine involution process (Okano and Tomizuka, 1987; Kähn, 1992; Rajamahendran et al., 1994).

Transvaginal ultrasound-guided oocyte aspiration (TUGA) was first developed for cattle by Pieterse et al. (1988). This technique provides for rapid, noninvasive access to the ovaries. TUGA allows collection of oocytes from donor cows for embryo production using a nonsurgical procedure (Pieterse et al., 1991b; Looney et al., 1994). Thus, this procedure can be an alternative to traditional embryo transfer programs for the production of cattle embryos (Pieterse et al., 1991b; Kruip et al., 1991). It has been proposed that repeated recovery of oocytes with this method could produce more embryos per year than by standard embryo transfer procedures (Kruip et al., 1994).

The TUGA procedure for oocyte collection has been used in cows during different phases of the estrous cycle (Pieterse et al., 1991a). Furthermore, this technique has been used for oocyte recovery in prepubertal calves (Brogliatti and Adams, 1996; Fry et al., 1998; Duby et al., 1996), pregnant cows (Meintjes et al., 1995a), infertile cows (Looney et al., 1994), cycling cows (Pieterse et al., 1991a; Kruip et al., 1991; Gibbons et al., 1995; Garcia and Salaheddine, 1998), postpartum beef cows (Caamaño, 1999; Perez et al., 2000; 2001a, 2001b), postpartum dairy cows (Caamaño, 1999), horses (Cook et al., 1992, Brück et al., 1992; Meintjes et al., 1995b; Squires, 1996), goats (Graff et al., 1999) and pigs (Bellow et al., 2001). This technique has also been applied in humans (Lenz et al., 1981; Dellenbach et al., 1985; Yee, 1988) and endangered species (Adams et al., 1991; Asa et al., 1998).

TUGA can be conducted once- or twice-a-week, and up to 3 months of pregnancy in lactating dairy and beef cows (Reinchenbach et al., 1993; Gibbons et al., 1994; Meintjes et al., 1995a). During these procedures no detrimental effects of follicle aspiration has been noted in cyclic, pregnant and postpartum cows (Kruip et al., 1994; Meintjes et al., 1995a; Perez et al., 2001a). Exogenous hormones, such as FSH and bovine somatotropin (bST) have been used to
increase follicular growth on the ovaries in heifer calves (Irvine et al., 1993; Brogliati et al., 1997; Hwang et al., 1997) and dairy cows (Looney et al., 1994; Ridell et al., 1997).

It is proposed that TUGA could be a useful reproductive tool to harvest oocytes from cattle during the postpartum period. In a preliminary report, this procedure has been used for oocyte collection in the late postpartum period (> 30 days) in dairy and beef cows (Caamaño, 1999). The potential application of this procedure in postpartum cows together with IVM, IVF and IVC might have a potential application producing extra calves when the cow is not biologically capable (Perez et al., 2000; 2001a). The objective of this study was to evaluate the effect of FSH for oocyte production in early postpartum dairy cows at day-25 and day-35 postpartum.

Materials and Methods

Experimental Animals

Twenty multiparous postpartum Holstein cows (3 to 4 years-of-age), with body condition scores ranging from 3.0 to 3.7 (1 = thin condition to 5 = fat condition), and milk production (mean ±SD) of 32.8 ± 7.2 kg/day (milked twice daily) were selected for this experiment during the late fall and winter months. Cows were fed with a balanced total-mixed ration consisting of corn silage, corn grain, alfalfa, dairy pellets, whole cottonseed and sodium bicarbonate. In addition, cows were placed on a bermudagrass pasture for ~2 hours each day. Follicle reduction was performed using a TUGA at day-15 postpartum. During this procedure, all follicles (>5 mm in diameter) were aspirated from both ovaries.

Experimental Design

The gonadotropin treated group (FSH treatment) (n=10) received a total of 32 mg of FSH (FSH-P, Follicle Stimulating Hormone Porcine; Lot No. 3090 with <6% LH concentration, Sioux Biochemical, Sioux Center, IA) administered (sc) once daily in descending doses over a 4-day period (Figure 4.1). The control group (no FSH treatment) (n=10) similarly received the same total volume of saline carrier vehicle (6.5 mL) in similar descending doses over a 4-day period.

Cows (n=20) were randomly assigned to one of four treatment groups in a 2 x 2 factorial arrangement where the main components were gonadotropin treatment (FSH and no FSH) and time postpartum of oocyte aspiration (day-25 and day-35 postpartum). These four treatment groups were: FSH with oocyte aspiration at day-25 postpartum (Treatment 1), saline vehicle with no FSH with oocyte aspiration at day-25 postpartum (Treatment 2), FSH with oocyte aspiration at day-35 postpartum (Treatment 3) and vehicle with no FSH with oocyte aspiration at day-35 postpartum (Treatment 4). The oocyte collections using a standard TUGA procedure were performed at day-25 and again at day-35 postpartum (each ~18 hours after the last dose of FSH or vehicle).
**Figure 4.1. Experimental procedure for postpartum dairy cows.**

**Oocyte Aspiration Procedure**

Cows were restrained in a stock chute for the transvaginal ultrasound-guided oocyte aspiration procedure. Each donor cow received 5 to 7 mL of lidocaine epidural injection (Lidocaine Hydrochloride 2%, Butler, Columbus, OH) to induce an anesthetic effect. To perform the aspiration of oocytes an Aloka 500-V real time ultrasound scanner (Corometrics Medical Systems, Inc., Wallingford, CT) was used with a 5 MHz array transducer. This transducer was attached to a 60-cm length plastic probe containing a stainless steel needle guide. A 17-gauge, 55-cm length stainless needle was used to aspirate the follicles. This needle was connected by tubing to an Em-Con® filter (Professional Embryo Transfer Supply, PETS, Canton, TX). The aspiration of follicles was performed with the aid of a regulated vacuum pump (Cook Veterinary Products, Australia), with a negative pressure of 70 mm Hg. The vacuum pressure was adjusted between each aspiration. After the ovary was visualized, the oocyte aspiration needle (previously introduced into the needle guide) was inserted through the vaginal wall and into the ovarian follicles. Negative pressure was applied as the needle entered into each follicle to enable recovery of the oocyte.

All the follicles >5 mm in diameter in each ovary were aspirated on day-15 postpartum. In this study, the collection medium consisted of Dulbecco’s phosphate-buffered saline (Gibco, Grand Island, NY) with 1% calf serum (Gibco-BRL, Life Technologies, Grand Island, NY), 5 units/mL of heparin (Elkins-Sinn, Inc., Cherry Hill, NJ) and 0.5 mg/mL of gentamicin (Gibco-BRL, Life Technologies, Grand Island, NY).
Oocytes collected on day-25 and day-35 were evaluated and divided into two groups on the basis of the presence and number of cumulus cell layers surrounding the oocytes: 1) oocytes that had more than two complete layers of cumulus cells were classified as “excellent” or “good” and 2) oocytes that had less than two complete layers of cumulus cells were given a “poor” classification. Oocytes were culture for 24 hours for in vitro maturation using Tissue Culture Medium-199 (TCM-199; Gibco-BRL, Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, Hi Clone Laboratories, Logan, UT) and 1 mg/mL of gentamicin at 39°C with an atmosphere of 5% CO₂ in humidified air.

**In Vitro Fertilization and In Vitro Culture**

Oocytes were fertilized after a 24-hour in vitro maturation period. Semen from a fertile Holstein bull (CSS 7H3429, INKA Rotate Adam-ET, No. 2044052) was thawed in 36°C water for 45 seconds and then placed in Brackett-Oliphant medium (Brackett and Oliphant, 1975). A 50 µL volume of sperm, with a concentration of 1 x 10⁶ sperm/mL was then placed into a 50 µL microdroplet of IVF medium (total of 100 µL in the fertilization microdroplet). Fertilization was performed at 39°C in a humidified incubator with 5% CO₂ during a 16-hour interval.

After 16 hours of incubation with sperm, oocytes were cultured in CR1aa medium with 4% BSA (Rosenkrans et al., 1994) for 7 days at 39°C in an atmosphere of 5% of CO₂ in air. Blastocyst formation was evaluated on both day-7 and day-8 after in vitro fertilization.

**Statistical Analysis**

The mean follicle production per donor, oocyte recovery per donor, oocytes per donor (excellent and good category) and embryo development data were evaluated using a completely randomized balanced design. Initially, 10 cows were randomly assigned to each of two exogenous treatments (FSH and no FSH). A mixed-effect linear model was fit to these data to perform the analysis of variance. The model statement including treatment (FSH and no FSH), time postpartum for oocyte collection (aspiration at day-25 and day-35 postpartum) and the interaction, with a significant difference set at the P<0.05 level. A multiple comparison analysis of the treatment LS means was performed with a Tukey’s adjustment test. All analyses were performed with the SAS® System for Microsoft Windows® 2001, SAS Institute, Inc., Cary, NC.

**Results**

The total overall number of follicles (>5 mm) per cow (mean ± SE) in both oocyte aspirations (at day-25 and day-35 postpartum) was 23.2 ± 1.9 follicles for the FSH-treated group (Figure 4.2). The total number of follicles (>5 mm) aspirated per cow from both oocyte collections was 17.4 ± 1.2 for the FSH-treated group. The number of oocytes recovered per cow from both
collections was 11.1 ± 0.7 (64% recovery rate). In the control group, the total overall number of follicles (>5 mm) per cow in both oocyte collections was 3.2 ± 0.3 follicles per cow. The total number of follicles aspirated per cow was 2.5 ± 0.3, resulting in 1.5 ± 0.2 oocytes recovered (60% recovery rate). The total number of follicles present on the ovaries, the total number of follicles aspirated and the total number of oocytes recovered per cow in both collections in the FSH-treated group were significantly greater when compared with those in the saline-treated control group (P<0.01). The overall average oocyte recovery rate; however, was not significantly different between the FSH and control treatment groups at 64% and 60%, respectively.

![Bar chart showing follicles, follicles aspirated, and oocytes per cow for FSH and control groups.](image)

**Figure 4.2.** Total overall follicles, follicles aspirated and oocytes produced per female from FSH-treated and control postpartum dairy cows.

When evaluating the results by day of oocyte collection, the number of follicles (>5 mm) per donor at day-25 postpartum for the FSH-treated group was 13 ± 1.9, of which 10.1 ± 1.4 follicles were aspirated, resulting in 6.1 ± 0.7 oocytes harvested (60% recovery rate) (Table 4.1). The saline-treated control group had 1.4 ± 0.2 follicles, of which 1.3 ± 0.2 follicles were aspirated and 0.8 ± 0.2 oocytes were recovered for a 62% recovery rate. All parameters for the 25-day
### Table 4.1. Mean (± SE) oocytes produced per female from FSH-treated and control dairy cows during the first oocyte aspiration at day-25 postpartum

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of follicles</th>
<th>No. of follicles aspirated</th>
<th>No. of oocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH*</td>
<td>13.0 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.1 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.1 ± 0.7&lt;sup&gt;a&lt;/sup&gt; (60%)</td>
</tr>
<tr>
<td>No FSH</td>
<td>1.4 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.8 ± 0.2&lt;sup&gt;b&lt;/sup&gt; (62%)</td>
</tr>
</tbody>
</table>

*One cow lost during the experiment due to a postpartum uterine infection. Data were not included in the analysis.

<sup>a,b</sup>Means within columns with different superscripts are significantly different (P<0.05).

### Table 4.2. Mean (± SE) oocytes produced per female from FSH-treated and control dairy cows during the second oocyte aspiration at day-35 postpartum

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of follicles</th>
<th>No. of follicles aspirated</th>
<th>No. of oocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH*</td>
<td>10.2 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.3 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0 ± 0.7&lt;sup&gt;a&lt;/sup&gt; (68%)</td>
</tr>
<tr>
<td>No FSH</td>
<td>1.8 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.7 ± 0.2&lt;sup&gt;b&lt;/sup&gt; (58%)</td>
</tr>
</tbody>
</table>

*One cow lost during the experiment due to a postpartum uterine infection. Data were not included in the analysis.

<sup>a,b</sup>Means within columns with different superscripts are significantly different (P<0.05).
The total number of excellent and good quality oocytes per donor in the day-25 and day-35 postpartum FSH-treated groups were significantly different (P<0.05) when compared with those of the control group, except for oocyte recovery rates (60% vs. 62%).

When evaluating the results by day of oocyte collection, the day-35 FSH-treated group had 10.2 ± 1.9 follicles per donor, of which 7.3 ± 0.9 follicles were aspirated and 5 ± 0.7 oocytes were recovered for a 68% recovery rate (Table 4.2). The saline-treated control group had 1.8 ± 0.3 follicles per female, of which 1.2 ± 0.3 follicles were aspirated and 0.7 ± 0.2 oocytes were recovered for a 58% recovery rate. All parameters for the day-35 FSH-treated group were significantly different (P<0.05) when compared with those of the control group, except for oocyte recovery rates (68% vs. 58%).

Overall, the total number of oocytes per collection with excellent and good quality scores was 3.5 ± 0.5 and 0.1 ± 0.1 for the FSH-treated and control groups, respectively. The number of excellent and good quality oocytes per collection was similar in the FSH-treatment group at day-25 postpartum (1st oocyte aspiration) and day-35 postpartum (2nd oocyte aspiration) with 3.7 ± 0.3 and 3.3 ± 0.9 oocytes, respectively (Figure 4.3).

As expected, the FSH-treated group resulted in more excellent and good quality oocytes per female (3.7 ± 0.3 and 3.3 ± 0.9 oocytes per donor) than cows in the control group (0.1 ± 0.1 and 0.2 ± 0.1 excellent and good quality oocytes per donor) on day-25 and day-35 postpartum,
respectively (Tabular data not presented). The number of poor quality oocytes per donor female tended to be lower from FSH-treated cows in the day-35 postpartum group than in the day-25 postpartum group (1.7 ± 0.3 and 2.7 ± 0.5 poor quality oocytes, respectively); however, these means were not significantly different.

The overall number of \textit{in vitro} embryos produced per cow was low for both the FSH-treated and control groups. The number of blastocysts per collection produced from the FSH-treated group was 0.8 ± 0.3, while no \textit{in vitro}-produced embryos developed in the control group. The blastocyst production rate was 1 ± 0.5 per donor for the FSH-treated group starting on day-25 postpartum and 0.7 ± 0.4 blastocysts per cow for donors starting on day-35 postpartum. The percentage of blastocysts produced from cleaved embryos per collection for the day-25 FSH-treated donors was 26% and 20% for the day-35 FSH-treated donor females.

Good quality blastocysts produced from the day-25 and day-35 FSH-treated cows were transferred (2 embryos per female) to 5 available beef recipients (7 days post-estrus), of which 2 of these recipients were diagnosed as pregnant at 35 days of gestation by transrectal ultrasonography. A Holstein calf was born from a FSH-treated Holstein cow, whose oocytes were harvested by TUGA at day-25 postpartum. This healthy calf was a male and weighed 54.5 kg (See Appendix A, Figure 7.2 and 7.3).

\textbf{Discussion}

Transvaginal ultrasound-guided oocyte aspiration has been successfully used on cows in different stages of their reproductive cycle. In this study, efforts were made to use the ultrasound-guided oocyte aspiration procedure in the early postpartum dairy cow. TUGA has proved to be a reliable method for oocyte retrieval from living donor cows. No detrimental side effects of follicle aspiration from this procedure in cows have been reported by using TUGA (Pieterse \textit{et al.}, 1989; Kruip \textit{et al.}, 1994). Collecting oocytes using this technique has not caused any detrimental effects in pregnant cows (Meintjes \textit{et al.}, 1995a). The results of this experiment also confirm that this method is a reliable and safe technique to obtain oocytes from donor cows during the early postpartum period.

Results of this study indicate that ovaries of dairy cows are capable of responding to exogenous FSH treatment to increase the growth of follicles beginning at day-25 postpartum. This technique has been used in a preliminary study on dairy cows after 30 days postpartum (Caamaño, 1999) and in beef cows in the early postpartum period in this laboratory (Perez \textit{et al.}, 2000, 2001a).

In the present study, the overall response to the FSH treatment at day-25 and day-35 postpartum was 23.2 follicles (>5 mm) developing per cow. These results were markedly
improved over those from a preliminary study in dairy cows reported by Caamaño (1999), where only 16.5 follicles per cow were reported after 30 days postpartum, with 90% of these classified as small-size follicles (<4 mm). It has been proposed that the follicular development response to exogenous FSH in postpartum dairy cows is based on the recruitment and stimulation of the follicle populations present shortly after parturition (Duffor and Roy, 1985; Spicer et al., 1986b; Savio et al., 1990). In the present study, the follicle production following the first FSH stimulation at day-25 postpartum was 13 follicles per cow. These results are similar to those found in beef cows of 14.6 follicles per cow at day-25 postpartum (Perez et al., 2000). Conversely, the control cows only produced 1.4 and 1.8 follicles at day-25 and day-35 postpartum, respectively. Similarly, Pieterse et al. (1991a) reported 1.6 follicles per cow per estrous cycle with an oocyte recovery rate of 55% with TUGA in nontreated cycling dairy cows.

An overall total of 11.1 oocytes were harvested from two collections from the 9 FSH-treated postpartum dairy cows in this study. In contrast, only an average of 1.5 oocytes was retrieved from similar cows in the control group from two collections at day-25 and day-35 postpartum. In this study, the number of oocytes recovered per donor was 6.1 and 5 for cows treated with FSH starting at day-25 and day-35 postpartum, respectively. Correspondingly, other researchers have reported an average of 7 oocytes recovered per donor in cycling FSH-treated dairy cows with twice-weekly aspirations (Bungartz et al., 1995) and 9 oocytes recovered per donor in nonlactating FSH-treated dairy cows in a single oocyte collection (Bousquet et al., 1999). Krüip et al. (1994) reported 8 oocytes harvested per female in nontreated dairy cows under a twice-a-week oocyte collection program using TUGA. Our results in obtaining oocytes from postpartum dairy cows are similar to the results obtained in cycling cows. This demonstrates that TUGA can be applied in the early postpartum period in dairy cows starting at day-25 postpartum.

Only 4 oocytes were recovered per cow after 30 days postpartum (54% recovery rate) has been reported in dairy cows with no gonadotropin stimulation (Caamaño, 1999). Similarly, 5 oocytes were recovered per cow (57% recovery rate) in FSH-treated dairy cows after 30 days postpartum (Caamaño, 1999). The percent of oocytes recovered from FSH-treated dairy cows at day-25 and day-35 postpartum in our study was 60% and 68%, similar to the 62% and 58% recovery rates for the control cows, respectively. These oocyte recovery rates; however, were similar to the range of 57% to 68.5% reported in later stage postpartum dairy cows (Caamaño, 1999), problem dairy cows (Looney et al., 1994), nontreated dairy cows (Krüip et al., 1994) and cycling dairy cows (Bungartz et al., 1995).

Using TUGA, it is possible to obtain oocytes from lactating cows in the postpartum period and to evaluate the overall oocyte quality. Perez et al. (2001) reported 46% excellent and good
quality oocytes harvested from beef cows during the first 20 days postpartum. Caamaño (1999) reported that only 25% of the dairy oocytes recovered in the late postpartum period were classified as being of excellent and good quality. These results support the proposal by Wallace et al. (1989), Schrick et al. (1993) and Inskeep (1995) that oocytes from early postpartum follicles are not responsible for the early infertility of the postpartum cow.

The overall number of excellent and good quality oocytes per collection recovered from FSH-treated dairy cows on day-25 and day-35 postpartum were 3.5 and 0.1 for the FSH-treated and control cows, respectively. The number was similar at day-25 and day-35 postpartum for the FSH-treated group with 3.7 and 3.3 oocytes per collection, respectively. Caamaño, (1999) reported a low number of oocytes of good quality per collection in FSH-treated dairy cows (1 oocyte per cow) at day-30 postpartum. In contrast, Perez et al. (2000) have reported that the number of excellent and good quality oocytes in the FSH-treated and control beef cows improved from the first oocyte aspiration at day-25 postpartum to the second oocyte aspiration at day-35 postpartum. It has become obvious that the quality of oocytes in various stages of the postpartum period has not been widely studied and certainly needs further investigation.

In this study, in vitro fertilization of oocytes obtained using TUGA in dairy cows resulted in only a limited number of embryos developing to the blastocyst stage. Overall, 60% of the FSH-treated donor cows produced at least one blastocyst/collection. The average blastocyst production in FSH-treated dairy cows at day-25 postpartum was 1 per female (26% of those that cleaved) and 0.7 blastocyst per cow (20% of those that cleaved) at day-35 postpartum. Caamaño (1999) reported a 7.9% blastocyst formation rate in dairy cows at day-30 postpartum; whereas, Bungartz et al. (1995) reported a 3.8% blastocyst rate in FSH-treated cycling dairy cows. Similar results in blastocyst production rates were reported by Perez et al. (2000, 2001a, 2001b), where 1.2 blastocysts per female were produced from postpartum beef cows harvested between day-5 and day-35 postpartum.

TUGA has been proposed to be an alternative to traditional embryo transfer programs for the production of cattle embryos (Kruip et al., 1991; Pieterse et al., 1991). We also propose that this approach could also be used to produce supplemental pregnancies from valuable dairy donor cows before the female starts cycling naturally for mating or AI. In our study, a supplemental calf was produced from oocytes harvested from a day-25 postpartum FSH-treated donor Holstein cow. Under typical dairy farm management at this station the oocyte-donor dairy cow produced two calves in a 12-month interval (example see Appendix A, Figures 7.2 and 7.3).

In summary, the present results indicate that early postpartum FSH-treated Holstein cows can be a source of oocytes of excellent and good quality at day-25 and day-35 postpartum for in
vitro embryo production programs. It is necessary to perform additional research using transvaginal ultrasound-guided oocyte aspiration in the early postpartum dairy cow and to improve the ovarian follicle stimulation with modified FSH treatment schedules.
CHAPTER V
EFFECT OF SOMATOTROPIN AND ENERGY SUPPLEMENTATION ON OOCYTE PRODUCTION FROM POSTPARTUM BEEF COWS 10 DAYS AFTER PARTURITION

Introduction

Transvaginal ultrasound-guided oocyte aspiration (TUGA) has been used widely to harvest bovine oocytes to be used in IVF programs (Pieterse et al., 1989, 1991b; Kruip et al., 1993; Rath, 1993; Kruip et al., 1994). TUGA was originally developed by Pieterse et al. (1989) and is the most commonly used method for oocyte recovery in cattle (Kruip et al., 1990; Pieterse et al., 1991b; Looney et al., 1994; Bungartz et al., 1995; Fry et al., 1998).

The TUGA is proposed as an alternative reproductive tool to produce oocytes during the postpartum period. These oocytes harvested by TUGA have been used in IVM, IVF and IVC programs to produce viable offspring. It has been proposed that TUGA could be used to produce embryos in postpartum cows prior to day-30 postpartum (Perez et al., 2000, 2001a). The potential production of oocytes, embryos, pregnancies and calves in early postpartum beef cows has not been thoroughly investigated.

The TUGA procedure has been used in a preliminary study in the late postpartum period (>30 days) in dairy and beef cows (Caamaño, 1999). The potential application of this procedure in postpartum cows together with IVM, IVF and IVC might have a potential application producing extra embryos and calves when the cow is not biologically capable (Perez et al., 2000, 2001a). This approach may provide an alternative reproductive method as an efficient source of oocytes for the production of IVF embryos before 30 days postpartum. Perez et al. (2000, 2001a) demonstrated that FSH-treated beef cows during the first 25 days after calving could be a source of good quality oocytes and embryos in the early postpartum period.

A twice daily FSH injection protocol (using decreasing daily doses) is the most commonly option for superovulatory responses in cattle in North America. However, the multiple injection approach can be a source of stress for cattle, especially when cows with calves are handled frequently during the early postpartum interval. Polyvinylpyrrolidone is a suitable solvent for prolonging the absorption of FSH given in a single injection. Polyvinylpyrrolidone has been used successfully as a vehicle for FSH-P for inducing superovulation in beef cattle (Smith et al., 1973). A single injection method can induce an acceptable superovulatory response when compared with a multiple FSH injection program that maintains a high plasma level of FSH (Smith et al., 1973; Yamamoto et al., 1994; Takedomi et al., 1995; Sugano and Shinogi, 1999).
The use of FSH along with bST has improved the follicular development and oocyte recovery rates in calves (Irvine et al., 1993; Hwang et al., 1997) and cows (Kirby et al., 1997a, 1997b; Ridell et al., 1997; Bols et al., 1998). Bovine somatotropin has been reported to have an overall stimulating effect on ovarian follicular activity (Gong et al., 1993, 1996), an effect that is likely mediated through the increased circulating levels of IGF1 (Gong et al., 1993).

Bovine somatotropin acts by increasing the total number of small-size follicles and the number of medium-size follicles available for gonadotropin stimulation (De la Sota et al., 1993; Bols et al., 1998). Bovine somatotropin has been reported to increase the number of small-size follicles (3 to 9 mm) in cattle during the first follicular wave in the postpartum period but does not increase the number of large-size follicles in the 10 to 15 mm or the >15 mm size categories (Lucy et al., 1993).

This increase in follicle development does not parallel the oocyte recovery rates (Fry et al., 1994) or the embryo recovery rates in cattle (Kuenher et al., 1993; Bols et al., 1998). Thatcher et al. (2001) reported no differences in the total number of oocytes/embryos per collection between bST and control beef cows. The follicular response was increased with bST treatments but was not changed by the plane of nutrition of the dairy cows. The number of good quality oocytes recovered and IVF-derived blastocysts produced in an earlier report were similar between bST-treated and control beef cows (Trip et al., 2000).

Nutrient intake before and after parturition has been shown to influence the interval from calving to first ovulation (Spitzer et al., 1995). It is well known that energy intake can influence the body condition scores of cows after calving. This effect alters the follicular growth pattern after calving, primarily in the number of medium-size and large-size (>10 mm) follicles at day-5 to day-17 postpartum in beef cattle (Ryan et al., 1994). Increments in dietary fat intake can increase the population of medium-size follicles (Ryan et al., 1992); however, a high energy diet might shift the responsiveness of ovarian follicles to bST toward smaller-size (6 to 9 mm in diameter) follicles to class 3 to 5 mm size follicles in dairy cattle (Lucy et al., 1993).

The objective of this study was to evaluate the response of early postpartum beef cows in an ultrasound-guided transvaginal oocyte aspiration program using polyvinylpyrrolidone as vehicle for the FSH in a single dose with energy supplementation and/or bovine somatotropin treatments.

**Material and Methods**

**Experimental Animals**

Thirty two multiparous crossbred nursing postpartum beef cows (3 to 7 years-of-age), in moderate body condition (body condition scores from 5 to 6; Richards et al., 1986) were used in
this experiment during the early spring of 2001. A total dose of 33 g of polyvinylpyrrolidone (PVP, K30, MWt 40,000) was dissolved in 100 mL of distilled water and sterilized by autoclaving. The mixture was then divided into aliquots of 10 mL each and stored at 4°C. Just before use, FSH (FSH-porcine, Sioux Biochemical, Sioux Center, IA) (50 mg) was dissolved in 1 mL of saline and mixed thoroughly with 10 mL of 30% PVP. A total dose of 50 mg of FSH was dissolved in 10 mL of PVP and administered (sc) in a single dose on day-6 postpartum (Figure 5.1).

**Experimental Design**

Cows were randomly assigned to one of four treatment groups using a 2 x 2 factorial arrangement to either an energy supplementation (ES) diet or no energy supplementation (no ES) diet and/or bovine somatotropin (bST) treatments or no bovine somatotropin treatments (no bST). The treatments were as follows: no ES + no bST (Treatment 1), no ES + bST (Treatment 2), ES + no bST (Treatment 3) and ES + bST (Treatment 4). Treatments 1 and 2 were applied to eight cows each; whereas, Treatments 3 and 4 were applied to 7 cows each. Cows in appropriate treatment groups (Treatments 3 and 4) received additional energy supplementation for 30 days prior to the expected calving dates. A single oocyte collection procedure using a standard transvaginal ultrasound-guided oocyte aspiration procedure was performed on day-10 postpartum (Figure 5.2).

**a. Energy supplementation**

Cows received an energy supplemented (ES) diet of 125% of the requirements of net energy (NE) that was balanced with level two of the National Research Council (NRC), Nutrient Requirements of Beef Cattle 7th Revised Edition (1996, National Academy Press. Washington, D.C.). The ingredients used in this supplemented diet were ground corn (93.2%), soybean meal (3.1%), limestone magnesium (2.5%), calcium phosphate (0.6%) and trace mineralized salt (0.6%).

All cows in the feeding (ES) treatments received an adaptation period to this diet starting with 25% of the energy supplemented diet for 7 days. This energy supplementation was then given 30 days before the expected day of parturition. The total amount of the supplemented diet was 7.3 kg per day per cow. The standard net energy requirement was 17.3 MCal/day. This energy requirement was increased by 25% over the daily requirements to 21.6 MCal/day. Cows were weighed at the beginning of the energy supplementation and again when starting the FSH treatment. Body condition scores (scores = 1 to 9) were recorded on all cows at the same intervals prior to feeding the supplemental diet and at the time of the FSH treatment.
Figure 5.1. FSH treatment protocol. A single dose of 50 mg of FSH in 10 mL of polyvinylpyrrolidone.
Cows in the ES treatment groups were maintained in a ryegrass pasture with access to meadow hay in a standing hay feeder. The supplemented diet was given daily at 10:00 a.m. using 6 bunk feeders. At the time of parturition, cows were removed from this pasture to another smaller pasture with no access to the supplemental feeding. Cows that were in the treatment groups not receiving extra energy supplementation (Treatments 1 and 2) were treated similarly but without energy supplementation, either prior to and following calving.

b. Bovine somatotropin treatment

The somatotropin treatment consisted of two injections of bST (Posilac, 500 mg of Sometribove Zinc, Monsanto, St. Louis, MO) subcutaneously (sc) in the post-scapular region (behind the shoulder) at day-3 and day-6 postpartum (Treatments 2 and 4)(Figures 5.2 and 5.3).

Oocyte Recovery and In Vitro Fertilization

All follicles on both ovaries (>5 mm in diameter) were aspirated (~96 hours after the single FSH injection). The collection medium consisted of Dulbecco’s phosphate-buffered saline (Gibco, Grand Island, NY) with 1% calf serum (Gibco-BRL, Life technologies, Grand Island, NY), 5 units/mL of heparin (Elkins-Sinn, Cherry Hill, NJ) and 0.5 mg/mL of gentamicin (Gibco-BRL, Life Technologies, Grand Island, NY).

Oocytes recovered were morphologically evaluated and then allotted to one of four classifications based on the presence and number of cumulus cell layers surrounding the oocytes: 1) oocytes that had more than 4 layers of compact cumulus cells were classified as “A”. Oocytes with 2 to 4 layers of cumulus cells were classified as “B”. Oocytes with a single layer of cumulus cells or denuded were classified as “C”. Oocytes with expanded cumulus cells were classified as “D” and oocytes with abnormal development were classified as “E”. Oocytes with classifications A and B were considered excellent and good quality oocytes, respectively. The oocytes were then incubated for 24 hours in TCM-199 containing 10% fetal bovine serum (FBS), 5 µg/mL of FSH, 10 µg/mL of LH and 0.5 mg/mL of gentamicin under mineral oil in a humidified atmosphere of 5% CO2 at 39°C for in vitro maturation.

Following a 24-hour maturation interval, oocytes were in vitro fertilized with 50 µL of semen with a concentration of 1 x 10^6 sperm/mL from a fertile Holstein bull (CSS 7H3429, INKA Rotate Adam-ET 2044052) and then incubated in 50 µL microdroplets of IVF medium (100 µL total volume) for 5 hours at 39°C in a 5% CO2 humidified incubator. After 5 hours of incubation the presumptive zygotes were cultured in CR1aa medium with 4% bovine serum albumin (BSA) (Rosenkrans and First, 1994).
Figure 5.2. Experimental procedure.

Figure 5.3. Somatotropin protocol for treatment at day-3 and day-6 postpartum in early postpartum beef cows.
Granulosa cells surrounding the zygotes were not removed after fertilization, subsequently forming a confluent monolayer during the in vitro culture in CR1aa medium with 4% BSA. The in vitro culture was conducted at 39°C in an atmosphere of 5% of CO₂ in air for up to 8 days. Embryo development was assessed at 48 hours after insemination for cleavage. Blastocyst formation rate was determined on both day-7 and day-8 after in vitro fertilization.

**Statistical Analysis**

The 2 x 2 factorial arrangement was a completely randomized unbalanced design with four treatment combinations. These treatments were: no ES + no bST (Treatment 1), no ES + bST (Treatment 2), ES + no bST (Treatment 3) and ES + bST (Treatment 4). A mixed-effect linear model was fit to this data to perform an analysis of variance. The importance of both ES and bST were examined as fixed main effects. Interaction of these two main effects was also checked for strict additivity. The experimental error term for this model was the random effect of an individual cow within a given treatment combination. A multiple comparison analysis of the treatment LS means was performed with a Tukey adjustment test. These post-hoc Tukey tests were used to establish which pairs of means were significantly different and different from each other. All analyses were executed with SAS (Statistical Analysis Software) programs (Copyright 2001, SAS Institute Inc., Cary, NC).

**Results**

Two cows were excluded from this experiment (1 cow from the ES + no bST treatment group and 1 cow from the ES + bST treatment group) because they had post-calving uterine infections. There were no differences in body weight between the feeding groups (ES + no bST and ES + bST) and nonfeed control groups (no ES + no bST and no ES + bST) at the beginning of the FSH treatment (564 kg vs. 536 kg, respectively). Body condition scores at the beginning of the experiment (37 days before calving) were between 5 and 6 for all cows in all treatment groups. The mean body condition scores did not change in Treatments 1 and 2 (no energy supplementation). However, the body condition score increased by the beginning of the FSH treatment (5 days after parturition) to between 6 and 7 for the cows in the energy supplementation group (Treatments 3 and 4).

A single oocyte aspiration was performed in each cow at day-10 postpartum. The total number (± SE) of follicles produced per donor using the FSH + PVP protocol was: 17.6 ± 2.9, 23 ± 2.1, 17.1 ± 2.3 and 12.3 ± 1.3 for the no ES + no bST, no ES + bST, ES + no bST and ES + bST treatment groups, respectively (Table 5.1) (Figure 5.4). When cows received bST + no ES the follicular development was increased to 23 ± 2.1 follicles per treated donor compared with cows in the bST + ES treatment group with 12.3 ± 1.3 follicles per treated donor. FSH-treated
cows that received no ES + bST had the greatest follicular development response of all the treatment groups (no ES + no bST, ES + bST and ES + no bST). The total number of follicles was 23 ± 2.1 with the number of follicles aspirated 19 ± 1.5, of which 14.8 ± 1.2 oocytes were recovered per cow (78% recovery rate). In contrast, cows that received ES + bST had the lowest follicular response of all the treatment groups with 12.3 ± 1.3 follicles per cow, from which 10.9 ± 1.1 follicles were aspirated, recovering 6.6 ± 0.8 oocytes (61% recovery rate). The follicle development response was significantly lower in cows that received ES + bST compared with the cows in the no ES + no bST, the ES + no bST and the no ES + bST treatment groups.

Cows in the no ES treatment group (Treatments 1 and 2) had a greater follicular development response per donor than cows in the ES treatment group (Treatments 3 and 4) with 20.3 ± 1.5 and 14.7 ± 1.6 follicles >5 mm in diameter, respectively (P<0.05) (Figure 5.5).

Table 5.1. The number of follicles and oocytes produced per collection from a single FSH dose at day-6 postpartum in lactating crossbred beef cows

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>n</th>
<th>No. of follicles</th>
<th>Follicles aspirated</th>
<th>No. oocytes ( % recovery)</th>
<th>Oocyte quality**</th>
<th>Blastocyst*** (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no ES + no bST</td>
<td>8</td>
<td>17.6 ± 2.9a</td>
<td>17.1 ± 2.9a</td>
<td>12.5 ± 2.4 (73)a</td>
<td>5.6 ± 1.4b</td>
<td>1.1 (27)</td>
</tr>
<tr>
<td>no ES + bST</td>
<td>8</td>
<td>23.0 ± 2.1a</td>
<td>19.0 ± 1.5a</td>
<td>14.8 ± 1.2 (78)a</td>
<td>9.2 ± 1.4a</td>
<td>2.1 (30)</td>
</tr>
<tr>
<td>ES + no bST</td>
<td>8*</td>
<td>17.1 ± 2.3a</td>
<td>16.0 ± 2.3a</td>
<td>10.1 ± 2.5 (63)a</td>
<td>4.9 ± 1.5b</td>
<td>1.1 (28)</td>
</tr>
<tr>
<td>ES + bST</td>
<td>8*</td>
<td>12.3 ± 1.3b</td>
<td>10.9 ± 1.1b</td>
<td>6.6 ± 0.8 (61)b</td>
<td>2.9 ± 1.5b</td>
<td>0.9 (28)</td>
</tr>
</tbody>
</table>

*A female in this group had uterine infection and was dropped from the experiment.  

a,b Means (± SE) within columns with different superscripts are significantly different (P<0.05).  
**Excellent and good quality oocytes.  
***Blastocysts from oocytes that cleaved.
Figure 5.4. Follicular response to a single injection of FSH dissolved in PVP in cows treated with energy supplementation (ES) prior to calving and/or with bovine somatotropin (bST) on day-3 and day-6 postpartum.

Figure 5.5. Follicular development response at day-10 postpartum in FSH-treated beef cows with energy supplementation (ES) prior to calving and/or with bovine somatotropin (bST) on day-3 and day-6 postpartum.
Cows that received bST treatment (Treatments 2 and 4) did not have an increased number of follicles per donor compared with cows that received no bST treatment (Treatments 1 and 3) with 17.4 ± 1.6 and 17.6 ± 1.6 follicles >5 mm in diameter, respectively.

In the energy supplemented group that was given bST, the total number of follicles produced per cow was 17.1 ± 2.3 follicles, of which 16 ± 2.3 follicles were aspirated recovering 10.1 ± 2.5 oocytes (63% recovery rate). The oocyte recovery rates for the treatment groups were not significantly different and the overall oocyte recovery rate was 69% for all the treatment groups at day-10 postpartum.

The total number of oocytes collected per donor was: 12.5 ± 2.4, 14.8 ± 1.2, 10.1 ± 2.5 and 6.6 ± 0.8 for the no ES + no bST, no ES + bST, ES + no bST and ES + bST treatment groups, respectively (Table 5.1). Cows that received no ES + no bST, no ES + bST and ES + no bST had significantly more oocytes retrieved per donor (12.5 ± 2.4, 14.8 ± 1.2, 10.1 ± 2.5) compared with the ES + bST group with 6.6 ± 0.8 oocytes per cow.

Cows in the no ES treatment groups (Treatments 1 and 2) had greater oocyte production (P<0.05) than cows receiving ES treatment (Treatments 2 and 4) with 13.6 ± 1.3 and 8.3 ± 1.4, respectively (Figure 5.6). Cows that received no bST (Treatments 1 and 3) had 11.3 ± 3 oocytes per cow compared with cows in the bST treatment group (Treatments 2 and 4) with 10.7 ± 1.4 oocytes per cow. In this study, bovine somatotropin did not increase the number of oocytes retrieved per donor in FSH-treated cows at day-10 postpartum.

The total number of excellent and good quality oocytes produced per donor was: 5.6 ± 1.4, 9.2 ± 1.4, 4.9 ± 1.5 and 2.9 ± 1.5 for the no ES + no bST, no ES + bST, ES + no bST and ES + bST treatment groups, respectively. Cows that received no ES + bST had greater excellent and good quality oocyte produced (P<0.05) with 9.2 ± 1.4 oocytes per cow than cows in the no ES + no bST, ES + no bST and ES + bST treatment groups with 5.6 ± 1.4, 4.9 ± 1.5 and 2.9 ± 1.5 excellent and good quality oocytes per cow, respectively (Figure 5.7). Bovine somatotropin in the no ES + bST increased (P<0.05) the number of excellent and good quality oocytes with 9.2 ± 1.4 oocytes compared with those cows under the no ES + no bST, ES + no bST and ES + bST with 5.6 ± 1.4, 4.9 ± 1.5 and 2.9 ± 1.5 oocytes per cow, respectively. The number of excellent and good quality oocytes was greater (P<0.05) in the no ES treatment group (Treatments 1 and 2) with 7.4 ± 1.4 oocytes compared with cows in the ES treatment group (Treatments 2 and 4) with 3.9 ± 1.5 oocytes. Cows in the ES treatment group (Treatments 1 and 4) had greater number (P<0.05) of undeveloped oocytes per cow with 3 ± 0.5 oocytes than the no ES treatment group (Treatments 2 and 4) with 0.6 ± 0.47 oocytes (Data not presented in tabular form).
Figure 5.6. Oocyte production response in FSH-treated beef cows with energy supplementation (ES) prior to calving and/or with bovine somatotropin (bST) on day-3 and day-6 postpartum.

Figure 5.7. Excellent and good quality oocytes produced from FSH-treated beef cows with energy supplementation (ES) prior to calving and/or with bovine somatotropin (bST) on day-3 and day-6 postpartum.
The total number of blastocysts produced per donor was: 1.1, 2.1, 1.1 and 0.9 for the no ES + no bST, no ES + bST, ES + no bST and ES + bST treatment groups, respectively. The blastocyst production rate from cleaved embryos was 27%, 30%, 28% and 28% for the no ES + no bST, no ES + bST, ES + no bST and ES + bST treatment groups, respectively.

**Discussion**

Twice daily FSH injections for 4 days have been the most common method to stimulate bovine follicular growth for embryo transfer programs (Schneider *et al*., 1980). Furthermore, daily single injections of FSH have been successfully used to stimulate follicular development in postpartum dairy and beef cows (Perez *et al*., 2000, 2001a). A single injection of FSH dissolved in PVP has been successful to induce superovulatory responses in cattle (Suzuki *et al*., 1994). In our study, the use of FSH dissolved in PVP was demonstrated to be an option for stimulating follicular growth in cattle donors for a transvaginal ultrasound-guided oocyte aspiration program. Although FSH + PVP has been used in cycling dairy cows (Yamamoto *et al*., 1994), results from the present study suggest that FSH + PVP could be also used in early postpartum beef cows.

The response of the ovaries to the FSH + PVP treatment in this study was 20.3 follicles per donor for the no energy supplementation treatment groups and 14.7 for the energy supplementation treatment groups, respectively. These results were similar to those found in superovulated cycling dairy cows (Suzuki *et al*., 1994; Yamamoto *et al*., 1994; Takedomi *et al*., 1995) where FSH with PVP maintained high levels of circulating FSH when administering a single FSH dose per female (Takedomi *et al*., 1995).

Results of the present study are in agreement with those obtained in early postpartum beef cows using multiple FSH doses with a follicular response of 19.4 follicles (>5 mm in diameter) per donor (Perez *et al*., 2000, 2001a). Furthermore, the results from our study are comparable with those made also using multiple doses of FSH in problem cows at the beginning of the estrous cycle (Looney *et al*., 1994) and in early pregnant beef cows (Meintjes *et al*., 1995a).

It has been proposed that growth of follicles after calving was influenced by precalving energy intake. Ryan *et al*. (1992) has reported that dietary fat increased the population of medium-size follicles in beef cattle. The present study reports differences in follicular development between the energy supplementation treatment groups. Cows in the no energy supplementation treatment groups had more follicles per cow than cows in the energy supplementation treatment groups with 20.3 and 14.7 follicles per cow, respectively.

Similarly, Perry *et al*. (1991) found that medium-size follicles in postpartum beef cows were unaffected by energy levels before and after parturition. In addition, Lucy *et al*. (1993) found that dietary treatment did not influence medium-size follicles in dairy cows before 25 days.
postpartum. In contrast, other studies reported an increase in follicular development in early postpartum dairy cows under energy supplementation diets (Beam et al., 1997). These changes may reflect an increased movement of small-size follicles into medium-size follicle categories. The concentration of cholesterol in plasma and high density lipoproteins in follicular fluid are increased under regimens of energy supplementation because energy supplementation is thought to affect directly the steroidogenesis process and then follicular development in cattle. In our study, follicular development response was not affected by a short-term increase of energy in the diet.

Kendrick et al. (1999) reported that high energy diets subsequently produced smaller follicles in dairy cows; however, dairy cows given high energy diets produced more good quality oocytes. In the present study, neither the oocyte production nor the oocyte quality was affected by supplemental energy feeding. This was likely due to the amount of energy in the diet fed or the length of time cows were fed the supplementary diet. Our results are not in agreement with those reported by Lonergan et al. (1994) where the increase in follicle size in crossbred beef heifers had a beneficial effect on oocyte quality.

In the current experiment, the smaller number of good quality oocytes in the energy treatment groups compared with the number of good quality oocytes in the no energy supplementation treatment groups resulted in a similar number of blastocysts produced per cow. Furthermore, the low number of follicles and oocytes recovered in the energy supplementation groups could be due to the duration of the diet, animal to animal variation and amount of the energy above the recommended value (125% of NRC in our study). Overall, the energy supplementation in the present study was likely not enough or feed for long enough to cause a detectable response in the donor cows.

Bovine somatotropin has been reported to stimulate ovarian follicular activity in cyclic mares (Cochran et al., 1999), dairy cows (Herrler et al., 1994; Lucy et al., 1993), Bos indicus cows (Buratini et al., 2000) and postpartum European breed beef cows (Pinto et al., 1996), with a response that is likely mediated through circulating levels of IGF1 (Gong et al., 1993; Pinto et al., 1996) after bST administration. In our study, bST did not affect the number of follicles produced with 17.4 and 17.6 follicles per cow for the no bST and bST treatment groups, respectively. However, bST increased the response in cows that did not receive energy supplementation treatment (P<0.05). In contrast, cows that received energy supplementation + bST produced fewer follicles following the FSH treatment than cows that received no ES + no bST, no ES + bST and ES + no bST treatments. Tripp et al. (2000) reported that bST increased the number of follicles, but this follicular growth was unchanged by the plane of nutrition of the
beef donor females. The number of oocytes recovered per donor in the present study was not different in cows that received bST compared with cows that did not receive bST.

Our results tended to agree with those reported by Fry et al. (1994) and Bols et al. (1998), where follicular growth in cows increased without a corresponding increase in oocytes recovered. Cows that received bST treatment had increased oocyte quality likely because bST enhanced the oocyte maturation, fertilization rates and granulosa cell proliferation (Savion et al., 1981; Monniaux et al., 1992; Thatcher et al., 2001). The overall average oocyte recovery rate (69%) per donor was in agreement with most of the studies using TUGA (Pieterse et al., 1989; Looney et al. 1994; Bungartz et al., 1995; Perez et al., 2001b).

In summary, a single dose of FSH + PVP and the transvaginal ultrasound-guided oocyte aspiration technique is an available method to produce a good follicular response, oocytes and embryos at day-10 postpartum. However, when cows received a diet of 125% of the net energy requirements for 30 days prior to parturition the follicular response was not increased over donor cows not receiving energy supplementation.
CHAPTER VI

EFFECT OF A SINGLE DOSE OF GnRH ON LH SECRETION IN EARLY POSTPARTUM BEEF COWS

Introduction

Gonadotropin releasing hormone (GnRH) is a decapetide that regulates both LH and FSH release in farm animals (Schally et al., 1971; Clarke, 1987; Gazal et al., 1998). GnRH has been used to induce the release of LH in the postpartum period in sheep (Foster and Crighton, 1973; Gregg et al., 1986; Gonzalez and Murphy, 1988), dairy cows (Britt et al., 1974; Fernandes et al., 1978; Foster et al., 1980), beef cows (Edwards et al., 1983; Jagger et al., 1987; D’Occhio et al., 1989) and calves (Rodriguez and Wise, 1991).

The postpartum period in cattle is characterized by a low frequency of pulsatile LH (Peters and Lamming, 1986). This pulsatile release of LH in postpartum cows is relatively low immediately after parturition (~0.5 ng/mL) (Echternkamp and Hansel, 1973; Arije et al., 1974; Gautier et al., 1982; Walters et al., 1982a; Williams et al., 1983; Jagger et al., 1987; Nett et al., 1988) and its occurrence increases near the time of the first ovulation (Rawlings et al., 1980; Webb et al., 1980). This low circulating LH concentration is primarily the result of infrequent pulsatile LH release and is associated with prolonged postpartum anestrus in suckled beef cows (Humphrey et al., 1976; Peters et al., 1981; Williams et al., 1982). In suckled beef cows, LH content of the anterior pituitary increased from levels at parturition to levels similar to those present in cyclic beef cows (Moss et al., 1985; Nett, 1987).

During late pregnancy the hypothalamic-hypophysial axis is suppressed by high circulating concentrations of progesterone and estradiol (Nett et al., 1988). These high circulating steroid concentrations inhibit the secretion of GnRH resulting in inadequate stimulation of pituitary gonadotrophs to maintain synthesis of LH after calving (Nett et al., 1988). Although the hypothalamus contains sufficient amounts of LHRH to stimulate release of LH from the anterior pituitary, its release is inhibited during the early postpartum period (Moss et al., 1985). This produces depletion of LH in the anterior pituitary gland and suppression of ovarian follicular activity that is then restored during the late postpartum period (Walters et al., 1982a; Williams et al., 1982; Schallenberger et al., 1984; Peters et al., 1985).

Exogenous GnRH can induce LH release in cycling cows (Walters et al., 1982b; Roberge et al., 1992). Injection of exogenous GnRH can be used to promote ovarian activity and ovulation in acyclic postpartum cows (Gillian et al., 1981; D’Occhio et al., 1989; Crowe et al., 1993) but induced corpora lutea most often have a short life span (Riley et al., 1981; Butcher et al., 1992).
There have been numerous attempts to increase the frequency of LH pulses and ovulation in postpartum cows using exogenous GnRH administration. This hormone has been administered in different ways (Yavas and Walton, 2000a), such as a single injection of GnRH, intermittent (pulsatile) injections of GnRH (Walters et al., 1982c; Edwards et al., 1983; Spicer et al., 1986a) and continuous infusion of GnRH (Lofstedt et al., 1981; Jagger et al., 1987; Lamming and McLeod, 1988).

GnRH in a single injection causes an increase in the LH surge and ovulation in dairy cows between day-10 to day-18 postpartum (Britt et al., 1974; Kesler et al., 1977; Fernandes et al., 1978; Kesler et al., 1978; Foster et al., 1980; Schallenberger et al., 1984). In beef cows, the same response usually occurs at day-21 to day-31 postpartum (Smith et al., 1979; Carter et al., 1980; Irvin et al., 1981; Pratt et al., 1982; Wattemann et al., 1982; Troxel et al., 1983, 1984). In contrast, responsiveness of the pituitary to GnRH has been reported not induce LH and ovulation before 20 days postpartum in beef cows (Webb et al., 1977; Fonseca et al., 1980; Irvin et al., 1981). The objective of this study is to determine if a single dose of GnRH could modify LH secretion at day-5 and day-30 postpartum in crossbred beef cows.

Materials and Methods

Experimental Animals

Twelve multiparous crossbred beef cows (3 to 6 years-of-age) with a body condition score between 5 to 7 (1 = emaciated and 9 = obese) (Richards et al., 1986) were randomly assigned to receive one of four treatments during the spring of 2001. The cows were maintained on a bermudagrass pasture.

Experimental Design

The cows were randomly assigned by days postpartum using a 2 x 2 factorial arrangement. The main factors were either the time of treatment during the postpartum interval (day-5 or day-30) or GnRH treatment (GnRH or no GnRH) (Figure 6.1). Treatments are as follows: day-5 + GnRH (Treatment 1), day-5 + no GnRH (Treatment 2), day-30 + GnRH (Treatment 3) and day-30 + no GnRH (Treatment 4). All the treated animals received a dose of GnRH (Cystorelin, Abbot Laboratories, North Chicago, IL) at 100 µg/animal intravenously or an equal volume of saline carrier vehicle.

Blood Samples

Blood samples were collected by jugular venipuncture at 15-minute intervals from 30 minutes prior to GnRH injection and at 15-minute intervals for up to 255 minutes after the GnRH injection (Figure 6.1). Blood was collected into heparinized tubes (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ) and place in an ice bath until centrifuged at 3,000 x g for 15
Figure 6.1. Experimental Procedure.
minutes at 4°C. Serum samples were frozen at -20°C until assayed for LH content. Serum LH was determined by radioimmunoassay (RIA) previously described by Thompson et al. (1993).

Statistical Analysis

Data were analyzed with analysis of variance using PROC MIX with the model including postpartum days (day-5 and day-30 postpartum) and hormonal treatment (GnRH or no GnRH). Statistical analysis was conducted using the Statistical Analysis System (SAS)® software. Data were analyzed for the area under the response curve using proc print and proc GLM.

Results

Circulating LH concentration mean (± SE) of cows in the 5-day + GnRH treatment group was below 1 ng/mL (0.17 ± 0.03 ng/mL) (Figure 6.2). Similarly, LH concentrations in the 5-day + no GnRH treatment group also had circulating concentrations of LH below 1 ng/mL (0.04 ± 0.01 ng/mL) (Figure 6.3). All blood samples collected before the GnRH challenge and those collected before the saline treatments (Treatments 1 and 2) had LH concentrations less than 0.6 ng/mL. The secretion of LH in response to the administration of GnRH at day-30 postpartum in beef cows (Treatment 3) is shown in Figure 6.4. The cows given a 100-µg challenge dose of GnRH at day-30 postpartum induced a significantly greater (P<0.05) LH release (2.1 ± 0.3 ng/mL) than the same treatment to cows on day-5 postpartum (0.17 ± 0.03 ng/mL). Cows that responded to GnRH at day-30 postpartum had a marked increase in LH concentrations within 15 minutes after the GnRH injection with a peak concentrations occurring at ~120 minutes. The mean concentration of LH in cows in the day-30 postpartum group not given GnRH was 0.25 ± 0.03 ng/mL (Figure 6.5). The mean LH concentrations for all four of the treatment groups were: 0.17 ± 0.3, 0.4 ± 0.01, 2.1 ± 0.28 and 0.25 ± 0.03 for the day-5 + GnRH, day-5 + no GnRH, day-30 + GnRH and day-30 + no GnRH treatment groups, respectively.

The mean (± SD) area under the curve (AUC) for the different treatment groups was: 3.0 ± 2.4, 0.7 ± 0.4, 37.6 ± 24.7 and 4.4 ± 1.4 for the day-5 + GnRH, day-5 + no GnRH, day-30 + GnRH and day-30 + no GnRH, respectively (Table 6.1; Figure 6.6). The day-30 + GnRH treatment group had significantly (P<0.05) more LH secreted following treatments than the cows in the day-5 + GnRH, day-5 + no GnRH and day-30 + no GnRH treatment groups (Figure 6.7). When comparing the response to GnRH between day-5 + GnRH and day-30 + GnRH postpartum treatment groups, the pituitary response was greater when the GnRH challenge was given to cows at day-30 postpartum.
Figure 6.2. LH profiles from GnRH-treated at day-5 postpartum beef cows (Cow Numbers: 3006, 3088 and 7036). Blood samples collected at 15-minute intervals.
Figure 6.3. LH profiles from saline-treated (no GnRH) day-5 postpartum beef cows (Cow Numbers: 547, 3003, 8859). Blood samples collected at 15-minute intervals.
Figure 6.4. LH profiles from GnRH-treated day-30 postpartum beef cows (Cow Numbers: 3100, 6001, 9171). Blood samples collected at 15-minute intervals.
Figure 6.5. LH profiles from saline-treated (no GnRH) day-30 postpartum beef cows (Cow Numbers: 15, 3074 and 4002). Blood samples collected at 15-minute intervals.
Figure 6.6. LH response patterns for beef cows treated with GnRH or saline on day-5 or on day-30 postpartum. Mean ± SD.
Table 6.1. Mean (± SD) of the area under the curve of the LH profile following a GnRH or a saline challenge in beef cows at day-5 and day-30 postpartum

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-day postpartum + GnRH</td>
<td>3.0 a</td>
</tr>
<tr>
<td>5-day postpartum + no GnRH</td>
<td>0.7 a</td>
</tr>
<tr>
<td>30-day postpartum + GnRH</td>
<td>37.6 b</td>
</tr>
<tr>
<td>30-day postpartum + no GnRH</td>
<td>4.4 a</td>
</tr>
</tbody>
</table>

\(^a,b^\)Means within columns with different superscripts are significantly different (P<0.05).
Figure 6.7. Effect of GnRH on LH levels following a GnRH challenge in early postpartum cows.
Discussion

GnRH has been used to induce LH release in beef cows during the postpartum period (~30 days after parturition) (Edwards et al., 1983; Jagger et al., 1987). In these studies, GnRH challenge did not induce a LH response in beef cows as early as 30 days postpartum. Plasma LH concentration has been reported to increase after GnRH treatment in beef cows at 45 days postpartum (Wettemann et al., 1982). In our experiment, the GnRH challenge induced LH release that started within 15 minutes after treatment in the day-30 postpartum cows and peak LH concentrations occurred ~120 minutes post-GnRH treatment. These observations were similar to those reported by Fernandes et al. (1978) in dairy cows ~day-20 postpartum, Wettemann et al. (1982) and Williams et al. (1989) in late postpartum beef cows after day-40 postpartum. We found either a weak or delayed response in one of the day-30 postpartum GnRH-treated cows. Although this can not be explained, it is likely due to individual animal differences.

It has been proposed that the pituitary is not able to respond to GnRH in the early postpartum period in beef cattle (Smith et al., 1979; Irvin et al., 1981; Pratt et al., 1982). This lack of response in these cases may be due to a reduction of LH stores in the pituitary in the early postpartum period resulting from high concentrations of placental and ovarian steroids such as progesterone and estrogens (Nett et al., 1988). In our experiment, the pituitary of day-5 postpartum beef cows only released detectable levels of LH in response to a standard GnRH challenge, resulting in LH concentrations remaining below 1 ng/mL. These low LH concentrations were similar to those found in nontreated beef cows at day-5 postpartum (~0.5 ng/mL) (Echternkamp and Hansel, 1973; Arije et al., 1974; Peters et al., 1981; Peters and Lamming, 1986). Response to single injection of GnRH on day-5 postpartum in beef cows in our experiment could be due to depletion of LH stores in the anterior pituitary (Nett et al., 1988).

Absence of LH pulses in the early postpartum period is likely not due to insufficient hypothalamic GnRH content (Moss et al., 1985). Nett et al. (1998) found that hypothalamic GnRH content was greater in postpartum cows than the GnRH content in cyclic cows. This high GnRH content in early postpartum beef cows may indicate that GnRH is being suppressed. In the bovine pituitary, this results in accumulation of FSH, suppression of FSH release, reduction in LH stores and suppression of ovarian follicular activity (Schallenberger et al., 1984; Nett et al., 1988; Crowe et al., 1998). As result, pulsatile release of LH is reduced and pituitary content of LH could be not sufficient for initiation of ovulation and normal-length estrous cycles during the early postpartum period.
Researchers have reported that a single injection of GnRH did not cause a LH surge before day 21 postpartum in beef cattle (Webb et al., 1977; Fonseca et al., 1980; Irvin et al., 1981). In contrast, there have been two reports published that indicate a single injection of GnRH induced an LH surge either at day-3 or day-5 postpartum in beef cows (3.2 ± 1.2 ng/mL) (Carter et al., 1980; Williams et al., 1982).

Pituitary responsiveness to LH release from a single injection of 100 µg of GnRH in the day-30 postpartum treatment group was clearly greater than the other treatment groups. These results are similar to those observed using a single GnRH injection given between day-21 and day-31 postpartum in beef cows (Troxel et al., 1980; Pratt et al., 1982; Wettemann et al., 1982; Troxel et al., 1983, 1984). This LH response is likely because the pulsatile LH release capability in mature beef cows has recovered by day-25 to day-32 postpartum (Lamming et al., 1981; Yavas and Walton, 2000b). Anterior pituitary LH stores have been found to gradually increase at day-20 postpartum in beef cattle (Wagner et al., 1969).

There have been numerous attempts to induce ovarian response with exogenous GnRH in the early postpartum period. It appears that the hypothalamus and pituitary have been responsible for this low LH release response to GnRH. Our results further indicate that the pituitary of the early postpartum beef cow (day-5 postpartum) is not responsive to a GnRH challenge. However, the ovary could be a key in the onset of the responsiveness to exogenous gonadotropins in the early postpartum period in beef cows.

Although research studies have focused on the effect of LH in the postpartum period, the effect of circulating FSH has not been extensively studied during the early postpartum period in cattle. Williams et al. (1983) found a suppression of pulsatile FSH release and a suppression of plasma FSH concentrations during the first 2 weeks after parturition. However, there is no evidence at this time to indicate that FSH is a limiting factor in the postpartum period (Walters et al., 1982a; Moss et al., 1985). It has been shown that exogenous FSH can stimulate the ovary to grow follicles in the early postpartum beef cow (Perez et al., 2000, 2001a); however, few studies have been attempted to understand the ovarian response to gonadotropins in the early postpartum period in beef cows.

This laboratory reported follicle production of 20, 19.6, 20.2, 17.6 and 14.6 follicles per female in FSH-treated beef cows at 5, 10, 15, 20 and 25 days after calving, respectively (Perez et al., 2000; 2001a). Our results indicate that the ovaries per se does not seem to be a limiting factor because they can respond to exogenous gonadotropin stimulation. The ovarian response to FSH could be due for the recruitment and stimulation of small follicles present in the ovaries in the early postpartum period. The follicular development during the early postpartum period
has been characterized as having developing small- and medium-size follicles in cattle (Wagner and Hansel, 1969; Stevenson and Britt, 1979; Spicer et al., 1986b; Savio et al., 1990). Results of this study indicate that LH response to a single injection of 100 µg of GnRH was significantly reduced in early postpartum cows 5 days after calving; however, the pituitary of cows at day-30 postpartum regains the ability to release LH in response to a GnRH challenge.

Being able to stimulate follicle growth with gonadotropins will allow greater use of transvaginal ultrasound guided oocyte collection and increase oocytes available for in vitro fertilization thus increasing the efficiency of the early postpartum cow. The ovary does not seem to be a limiting factor because it can respond to exogenous gonadotropin stimulation.
SUMMARY AND CONCLUSIONS

Embryo transfer and transvaginal ultrasound-guided oocyte aspiration have been used in assisted reproductive in cattle. This new technology has been proposed to be successful in oocyte and embryo production in the early postpartum period. In our studies TUGA has been successfully used in the early postpartum period of dairy and beef cattle to harvest oocytes for use in in vitro fertilization programs. Furthermore, oocyte aspirations were performed as early as day-5 postpartum. Most of the oocyte aspirations in these series of experiments were performed during the time of normal uterine involution, which is usually completed by day-35 postpartum. Even though, uterus was in the process of involution, manipulation of the reproductive tract to aspirate the ovarian oocytes was performed without any technical problems. In these studies, results indicate that ovaries from early postpartum beef cows are capable to respond to an exogenous FSH treatment almost immediately after parturition.

In the Experiment I, the objective was to harvest oocytes from early postpartum FSH-treated beef cows with two oocyte aspirations at day-25 and day-35 postpartum. In this experiment the FSH treatment consisted of a total of 32 mg of FSH administered over a 4-day interval. The average number of oocytes recovered per cow across collections was 14.7, with recovery rate of 63%.

In the Experiment II, the objective was to evaluate follicle development and oocyte production of beef cows treated with FSH shortly after calving. A single oocyte collection was performed on FSH-treated cows at day-5, day-10, day-15 and day-20 postpartum. Overall the number of follicles, number of oocytes aspirated, percent of recovery rate, number of oocytes that cleaved, number of blastocysts developing from cleaved embryos and percent blastocyst produced per donor was: 19.4, 8.8, 64%, 4.3, 1.4 and 32.3%, respectively, for females aspirated between day-5 and day-20 postpartum. Although the oocytes recovered per cow were lower shortly after calving the recovery rates were similar in females aspirated later during the postpartum interval.

In the Experiment III, the objective was to harvest oocytes from early postpartum FSH-treated dairy cows with two oocyte aspirations at day-25 and day-35 postpartum. In this experiment the FSH treatment consisted of the same a total of 32 mg of FSH administered over a 4-day interval as beef cows. The average number of oocytes recovered per cow was 11.1 with a recovery rate of 64%. The lactating dairy donor cow on the average produced less oocytes per donor that the beef donor females aspirated at the same time during the postpartum interval.
In the Experiment IV, the objective was to evaluate the response of FSH-treated early postpartum beef cows using polyvinylpyrrolidone as the vehicle for the FSH in a single dose along with pre-calving energy supplementation and/or bovine somatotropin treatments. A single oocyte aspiration was performed on day-10 postpartum. Unexpectedly cows that did not receive any energy supplement prior calving had a greater oocyte production per female than cows given an energy supplemented diet prior to calving, with 13.6 and 8.4 oocytes per donor, respectively. Cows that received exogenous somatotropin treatments prior to FSH had 10.7 oocytes per cow, while cows not receiving somatotropin treatments prior to FSH produced 11.3 oocytes per cow. In this experiment, feeding cows with an energy supplemented diet for 30 days prior to calving appeared to decrease follicle development at day-10 postpartum in FSH-treated cows. The reason for this outcome can not be explained at this time. Both energy supplementation and somatotropin were expected to increase the number of follicles available for aspiration.

The FSH protocol used in the cows in Experiments I, II and III was a 4-day treatment with a daily FSH injections. This daily management in beef cows clearly caused animal stress because the calves were separated daily at the time of FSH injection. Results from the Experiment IV suggest that a single dose of 50 mg of FSH in PVP could be successfully used in the early postpartum beef cows. The use of a single injection of FSH greatly reduced animal handling stress while being able to stimulate follicular growth in beef cows treated as early as day-6 postpartum. Donor cows in this experiment had an average of 17.5 follicles per cow.

In addition, early FSH-treated postpartum beef cows were found to be a source of good quality oocytes. In these experiments ~40% of the oocytes collected in the early postpartum period (<30 days) were of excellent and good quality. These results indicate that good quality oocytes can be obtained shortly after parturition in the early postpartum cow. Furthermore, these oocytes could be fertilized and subsequently developed into embryos using conventional IVF methods. Oocytes harvested in the early postpartum period apparently have competency to develop viable embryos. Generally, one excellent to good quality blastocyst was produced per collection during the early postpartum period. Individual blastocysts were nonsurgically transferred to 9 available beef females, resulting in a 77% pregnancy rate and live calves were born.

These results demonstrate that good quality oocytes for blastocyst production can be produced from FSH-treated cows early (<30 days) during the postpartum period.
In the final experiment (Experiment V) the objective was to verify if a single GnRH challenge dose could modify LH secretion in beef cows at day-5 and day-30 postpartum. Results of this study indicated that LH response to a single injection of 100 µg of GnRH was significantly reduce in early postpartum cows 5 days after calving; however, the pituitary of cows at 30-days postpartum regained the ability of release LH in response to exogenous GnRH.

Our results further indicate that the ovaries per se are not the limiting factor in postpartum follicular development because the postpartum ovaries were readily stimulated following the administration of FSH. Being able to directly stimulate follicle growth with gonadotropins will allow greater use of transvaginal ultrasound-guided oocyte collection and increase oocytes available for in vitro fertilization thus, offering the opportunity for increasing reproductive efficiency in the early postpartum cow.
REFERENCES


collection in 10 to 16 weeks of age calves. Theriogenology 43: 177 (Abstr.).

Brück, I. and T. Greve. 1994. Transvaginal ultrasound-guided aspiration of follicular fluid in the
mare. Theriogenology 41: 170 (Abstr.).

Brück, I., K. Raun, B. Synnestvedt and T. Greve. 1992. Follicle aspiration in the mare using a

Brück, I., B. Synnestvedt and T. Greve. 1997. Repeated transvaginal oocyte aspiration in

via follicular aspiration aided by ultrasound with or without gonadotropin pretreatment
and in different reproductive stages. Theriogenology 43: 667-675.

and treatment with recombinant bovine somatotropin (bST) on ovarian follicular
development in nelore (Bos indicus) heifers. Theriogenology 54: 421-431.

Inskeep. 1992. Maintenance of pregnancy in postpartum beef cows that have short-

Ph.D. Dissertation, Iowa State University. Ames, IA.


Callesen, H.T.G. and R. Christenson. 1987. Ultrasonically guided aspiration of bovine follicular
oocytes. Theriogenology 27:217 (Abstr.).

hypothalamo-pituitary gonadotrophic axis of suckled and nonsuckled dairy cows

releasing hormone and calf removal on pituitary-ovarian function and reproductive


Jr. and R.A. Godke. 1999. The effects of equine somatotropin (eST) on follicular
development and circulating plasma hormone profiles in cyclic mares treated during


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APPENDIX A

OOCYTE AND EMBRYO PRODUCTION IN DARY COWS

Treatment: FSH

Cow No. 1574

Date of calving: 11-01-2000             25 days postpartum

Oocyte Aspiration

<table>
<thead>
<tr>
<th>Ovary size</th>
<th>Follicles</th>
<th>Follicles aspirated</th>
<th>Oocytes recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>right: 4.5 cm</td>
<td>right: 15</td>
<td>right: 9</td>
<td>15</td>
</tr>
<tr>
<td>left: 4.5 cm</td>
<td>left: 10</td>
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Oocyte recovery rate: 60%

Oocyte Quality Grades

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<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
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<tr>
<td>n =</td>
<td>12</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
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Embryo Production

<table>
<thead>
<tr>
<th>n</th>
<th>2-cell</th>
<th>16-cell</th>
<th>Blastocysts</th>
</tr>
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<tbody>
<tr>
<td>13</td>
<td>8</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 7.1. Oocyte production of a dairy cow (No. 1574) 25 days after parturition (Transvaginal ultrasound-guided oocyte aspiration from FSH-treated postpartum dairy cows).
Figure 7.2. Pregnancy diagnosis of an embryo recipient No. 6622 carrying a Holstein embryo from the donor No. 1574 (Transvaginal ultrasound-guided oocyte aspiration from FSH-treated postpartum dairy cows).
Figure 7.3. Holstein calf from a 25-day postpartum FSH-treated Holstein cow No. 1574 (Transvaginal ultrasound-guided oocyte aspiration from FSH-treated postpartum dairy cows).
Figure 7.4. Oocytes from a FSH-treated dairy cow (No. 3132) at 25 days postpartum (Transvaginal ultrasound-guided oocyte aspiration from FSH-treated postpartum dairy cows).
Figure 7.5. Energy supplementation on beef cows 30 days prior to parturition (Effect of somatotropin and single FSH-dose on oocyte production from post-partum beef cows 10 days after parturition).
Table 7.1. Preparation of the bovine oocyte maturation medium

Maturation Medium

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Catalogue No.</th>
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<tr>
<td>TCM-199</td>
<td>9.000 mL</td>
<td>Gibco 12340-030</td>
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<tr>
<td>FSH stock</td>
<td>0.020 mL</td>
<td>Sigma F-2293</td>
</tr>
<tr>
<td>LH stock</td>
<td>0.125 mL</td>
<td>Sigma L-9773</td>
</tr>
<tr>
<td>Serum</td>
<td>1.0 mL</td>
<td>Hyclone</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.010 mL</td>
<td>Gibco 15750-060</td>
</tr>
<tr>
<td>E₂</td>
<td>0.010 mL</td>
<td>Sigma E-2257</td>
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Hormone Stock Preparations

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<tr>
<th>Hormone</th>
<th>Amount and Description</th>
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<tbody>
<tr>
<td>FSH</td>
<td>1 vial contained 490 units (~35 mg)</td>
</tr>
<tr>
<td></td>
<td>Reconstitute with 14 mL of TCM-199 to give a 2.5 mg/mL of stock (35 IU/mL)</td>
</tr>
<tr>
<td></td>
<td>Aliquot in 50 µl portions and store at -80°C.</td>
</tr>
<tr>
<td></td>
<td>Add 0.020 mL of the stock to 10 mL maturation medium to give 5 µg/mL (0.7 IU/mL)</td>
</tr>
<tr>
<td>LH</td>
<td>1 vial contains 30,000 units (~8 mg)</td>
</tr>
<tr>
<td></td>
<td>Reconstitute with 10 mL of TCM-199 to give a 0.8 mg/mL stock (3,000 IU/mL)</td>
</tr>
<tr>
<td></td>
<td>Aliquot in 200 µl portions and store at -80°C.</td>
</tr>
<tr>
<td></td>
<td>Add 0.125 mL to 10 mL of maturation medium to give 10 µg/mL (375 IU/mL).</td>
</tr>
<tr>
<td>E₂</td>
<td>Add 10 mg estradiol to 10 mL of 95% EtOH to give a 1 mg/mL of the stock.</td>
</tr>
<tr>
<td></td>
<td>store at -80°C.</td>
</tr>
</tbody>
</table>
Table 7.2. Fertilization medium stock solutions

BO - A solution

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>4.3092 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.1974 g</td>
</tr>
<tr>
<td>CaCl$_2$ 2H$_2$O</td>
<td>0.2171 g</td>
</tr>
<tr>
<td>NaH$_2$PO$_4$ 2H$_2$O</td>
<td>0.0840 g</td>
</tr>
<tr>
<td>MgCl$_2$ 6H$_2$O</td>
<td>0.0697 g</td>
</tr>
<tr>
<td>Distilled, deionized water, Milli-Q</td>
<td>500 mL</td>
</tr>
<tr>
<td>Phenol red (0.5%)</td>
<td>0.1 mL</td>
</tr>
</tbody>
</table>

BO - B solution

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaHCO$_3$</td>
<td>2.5873 g</td>
</tr>
<tr>
<td>Distilled, deionized water, Milli-Q</td>
<td>200 mL</td>
</tr>
<tr>
<td>Phenol red (0.5%)</td>
<td>0.04 mL</td>
</tr>
</tbody>
</table>

Keep in the incubator at 39°C for at least 3 hours before use
### Table 7.3. Preparation of CR1aa medium (Rosenkrans and First, 1994)

#### Stock

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>0.6703 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.0231 g</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>0.2201 g</td>
</tr>
<tr>
<td>Pyruvic acid (P-2256 or P-4562)</td>
<td>0.0044 g</td>
</tr>
<tr>
<td>L(+) Lactic acid (L-4388)</td>
<td>0.0546 g</td>
</tr>
<tr>
<td>Glycine (G-8790)</td>
<td>0.0039 g</td>
</tr>
<tr>
<td>L-Alanine (A-7469)</td>
<td>0.0045 g</td>
</tr>
<tr>
<td>Distilled, deionized water, Milli-Q</td>
<td>100 mL</td>
</tr>
<tr>
<td>Phenol red (0.5% P 0290)</td>
<td>200 µL</td>
</tr>
</tbody>
</table>

#### Before use

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock</td>
<td>9.5 mL</td>
</tr>
<tr>
<td>BME (X50; b 6766)</td>
<td>200 µL</td>
</tr>
<tr>
<td>MEM (X100; M 7145)</td>
<td>100 µL</td>
</tr>
<tr>
<td>FBS</td>
<td>500 µL</td>
</tr>
<tr>
<td>Gentamicin (50 mg/mL)</td>
<td>10 µL</td>
</tr>
<tr>
<td>L-glutamine (G-5763)</td>
<td>0.00146 g</td>
</tr>
<tr>
<td>B SA (FAF; A 7511)</td>
<td>0.03 g</td>
</tr>
<tr>
<td>Insulin</td>
<td>10 µL</td>
</tr>
</tbody>
</table>

Filter
IN VITRO FERTILIZATION PROCEDURE

Pyruvic acid (P5280) = 0.00685 g

BO - A = 38 mL
BO - B = 12 mL
GENTAMICIN
HEPARIN (10,000 IU)

---

Add 25 mL

BO - CAFFEINE
Caffeine 0.0243 g
(Caffeine sodium C-4144)

Add 5 mL

BSA - BO (0.6%)
BSA = 0.03 g
To filter
Incubator (15-20 min)

Add 10 mL

BSA - BO (0.3%)
BSA = 0.03 g

---

This amount depends on the semen counting

Add 9-10 mL centrifuge for 6 min (500 g)
Add 9-10 mL centrifuge for 6 min

SEMEN

Add 2 mL BSA - BO 6%

Add 2 mL of BO - Caffeine

Wash the oocytes

---

10 oocytes/50 µL drops

Figure 7.6. *In vitro* fertilization protocol.
Table 7.4. FSH treatment protocol for oocyte donor cattle used in experiments:
Transvaginal ultrasound-guided oocyte aspiration from FSH-treated postpartum beef cows; Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving and Transvaginal ultrasound-guided oocyte aspiration from FSH-treated postpartum dairy cows

<table>
<thead>
<tr>
<th>Follicle Stimulating Hormone from Porcine Pituitary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sioux Biochemical, 204 Third Street N.W. Sioux Center, IA.</td>
</tr>
<tr>
<td>Lots No. 3091, 3092</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LH contamination was 3 to 6%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
</tr>
<tr>
<td>2.3 mL</td>
</tr>
<tr>
<td>11.5 mg</td>
</tr>
</tbody>
</table>
Table 7.5. Energy supplementation diet (125% of the NE) for crossbred beef cows

<table>
<thead>
<tr>
<th>Percent</th>
<th>Ingredient</th>
<th>Amount (kg/lb)/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>93.2</td>
<td>Corn ground</td>
<td>6.8/15</td>
</tr>
<tr>
<td>3.1</td>
<td>Soybean meal</td>
<td>0.2/0.5</td>
</tr>
<tr>
<td>2.5</td>
<td>Limestone magnesium</td>
<td>0.2/0.4</td>
</tr>
<tr>
<td>0.6</td>
<td>Calcium phosphate</td>
<td>0.05/0.1</td>
</tr>
<tr>
<td>0.6</td>
<td>Trace mineralized salt</td>
<td>0.05/0.1</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>7.3/16.1/day</td>
</tr>
</tbody>
</table>
APPENDIX B

OOCYTE AND EMBRYO PRODUCTION IN BEEF COWS

Treatment: FSH
BCS: 6.0

Cow No. 3036

Date of calving: 03-11-2000  5 days postpartum

Oocyte Aspiration

<table>
<thead>
<tr>
<th>Ovary size</th>
<th>Follicles</th>
<th>Follicles aspirated</th>
<th>Oocytes recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>right: 4.0 cm</td>
<td>right: 10</td>
<td>right: 8</td>
<td></td>
</tr>
<tr>
<td>left: 4.0 cm</td>
<td>left: 10</td>
<td>left: 2</td>
<td>4</td>
</tr>
</tbody>
</table>

>30 immature oocytes

Oocyte recovery rate: 40%

Oocyte Quality Grades

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = -</td>
<td>1</td>
<td>-</td>
<td>3</td>
<td>-</td>
</tr>
</tbody>
</table>

Embryo Production

<table>
<thead>
<tr>
<th>n</th>
<th>2-cell</th>
<th>16-cell</th>
<th>Blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 8.1. Oocyte and embryo production from donor cow No. 3036 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH  
BCS: 6.0

Cow No. 2063

Date of calving: 04-03-2000 5 days postpartum

Oocyte Aspiration

<table>
<thead>
<tr>
<th>Ovary size</th>
<th>Follicles</th>
<th>Follicles aspirated</th>
<th>Oocytes recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>right: 3.0 cm</td>
<td>right: 12</td>
<td>right: 10</td>
<td></td>
</tr>
<tr>
<td>left: 3.0 cm</td>
<td>left: 12</td>
<td>left: 8</td>
<td>11</td>
</tr>
</tbody>
</table>

>30 immature oocytes

Oocyte recovery rate: 61%

<table>
<thead>
<tr>
<th>Oocyte Quality Grades</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>n = 6</td>
</tr>
</tbody>
</table>

Embryo Production

<table>
<thead>
<tr>
<th></th>
<th>2-cell</th>
<th>16-cell</th>
<th>Blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Figure 8.2. Oocyte and embryo production from donor cow No. 2063 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH  
BCS: 6.0

Cow No. 611

Date of calving: 03-19-2000  5 days postpartum

Oocyte Aspiration

<table>
<thead>
<tr>
<th>Ovary size</th>
<th>Follicles</th>
<th>Follicles aspirated</th>
<th>Oocytes recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>right: 2.0 cm</td>
<td>right: 5</td>
<td>right: 5</td>
<td></td>
</tr>
<tr>
<td>left: 2.0 cm</td>
<td>left: 3</td>
<td>left: 3</td>
<td>6</td>
</tr>
</tbody>
</table>

Oocyte recovery rate: 75%

<table>
<thead>
<tr>
<th>Oocyte Quality Grades</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>n =</td>
</tr>
</tbody>
</table>

Embryo Production

<table>
<thead>
<tr>
<th>n</th>
<th>2-cell</th>
<th>16-cell</th>
<th>Blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 8.3. Oocyte and embryo production from donor cow No. 611 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH  
BCS: 6.0

Cow No. 9065

date of calving: 03-08-2000  5 days postpartum

Oocyte Aspiration

<table>
<thead>
<tr>
<th>Ovary size</th>
<th>Follicles</th>
<th>Follicles aspirated</th>
<th>Oocytes recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>right: 4.0 cm</td>
<td>Right: 16</td>
<td>right: 12</td>
<td></td>
</tr>
<tr>
<td>left: 3.0 cm</td>
<td>left: 14</td>
<td>left: 8</td>
<td>13</td>
</tr>
</tbody>
</table>

Oocyte recovery rate: 65%

<table>
<thead>
<tr>
<th>Oocyte Quality Grades</th>
<th>n = -</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
</table>

Embryo Production

<table>
<thead>
<tr>
<th>n</th>
<th>2-cell</th>
<th>16-cell</th>
<th>Blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>7</td>
<td>7</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 8.4. Oocyte and embryo production from donor cow No. 9065 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH
BCS: 6.0

Cow No. 7063

Date of calving: 02-16-200                  5 days postpartum

Oocyte Aspiration

<table>
<thead>
<tr>
<th>Ovary size</th>
<th>Follicles</th>
<th>Follicles aspirated</th>
<th>Oocytes recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>right: 3.0 cm</td>
<td>right: 11</td>
<td>right: 6</td>
<td></td>
</tr>
<tr>
<td>left: 3.0 cm</td>
<td>left: 7</td>
<td>left: 2</td>
<td>4</td>
</tr>
</tbody>
</table>

Oocyte recovery rate: 50%

<table>
<thead>
<tr>
<th>Oocyte Quality Grades</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>n = 1</td>
</tr>
</tbody>
</table>

Embryo Production

<table>
<thead>
<tr>
<th>N</th>
<th>2-cell</th>
<th>16-cell</th>
<th>Blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>7</td>
<td>7</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 8.5. Oocyte and embryo production from donor cow No. 7063 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH
BCS: 6.0

Cow No. 856

Date of calving: 03-16-2000                      10 days postpartum

Oocyte Aspiration

<table>
<thead>
<tr>
<th>Ovary size</th>
<th>Follicles</th>
<th>Follicles aspirated</th>
<th>Oocytes recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right: 4.0 cm</td>
<td>Right: 10</td>
<td>Right: 10</td>
<td>12</td>
</tr>
<tr>
<td>Left: 4.0 cm</td>
<td>Left: 12</td>
<td>Left: 8</td>
<td></td>
</tr>
</tbody>
</table>

Oocyte recovery rate: 66.6%

Oocyte Quality Grades

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>3</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Embryo Production

<table>
<thead>
<tr>
<th>n</th>
<th>2-cell</th>
<th>16-cell</th>
<th>Blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>9</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

Figure 8.6. Oocyte and embryo production from donor cow No. 856 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH  
BCS: 6.0  

Cow No. 7048  

Date of calving: 03-22-2000  
10 days postpartum

Oocyte Aspiration

<table>
<thead>
<tr>
<th>Ovary size</th>
<th>Follicles</th>
<th>Follicles aspirated</th>
<th>Oocytes recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>right: 3.0 cm</td>
<td>right: 8</td>
<td>right: 8</td>
<td>11</td>
</tr>
<tr>
<td>left: 3.0 cm</td>
<td>left: 7</td>
<td>left: 7</td>
<td></td>
</tr>
</tbody>
</table>

Oocyte recovery rate: 73%

<table>
<thead>
<tr>
<th>Oocyte Quality Grades</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Embryo Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>11</td>
</tr>
</tbody>
</table>

Figure 8.7. Oocyte and embryo production from donor cow No. 7048 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH  
BCS: 6.0

Cow No. 7018  
Date of calving: 02-14-2000  
10 days postpartum

Oocyte Aspiration

<table>
<thead>
<tr>
<th>Ovary size</th>
<th>Follicles</th>
<th>Follicles aspirated</th>
<th>Oocytes recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>right: 3.0 cm</td>
<td>right: 10</td>
<td>right: 7</td>
<td>7</td>
</tr>
<tr>
<td>left: 3.0 cm</td>
<td>left: 7</td>
<td>left: 7</td>
<td>8</td>
</tr>
</tbody>
</table>

Oocyte recovery rate: 57%  

<table>
<thead>
<tr>
<th>Oocyte Quality Grades</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>n =</td>
</tr>
</tbody>
</table>

Embryo Production

<table>
<thead>
<tr>
<th>n</th>
<th>2-cell</th>
<th>16-cell</th>
<th>Blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 8.8. Oocyte and embryo production from donor cow No. 7018 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH
BCS: 6.0

Cow No. 857

Date of calving: 03-10-2000  10 days postpartum

Oocyte Aspiration

<table>
<thead>
<tr>
<th>Ovary size</th>
<th>Follicles</th>
<th>Follicles aspirated</th>
<th>Oocytes recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>right: 3.0 cm</td>
<td>right: 7</td>
<td>right: 6</td>
<td></td>
</tr>
<tr>
<td>left: 3.0 cm</td>
<td>left: 7</td>
<td>left: 4</td>
<td>5</td>
</tr>
</tbody>
</table>

Oocyte recovery rate: 50%

<table>
<thead>
<tr>
<th>Oocyte Quality Grades</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>n = -</td>
</tr>
</tbody>
</table>

Embryo Production

<table>
<thead>
<tr>
<th>n</th>
<th>2-cell</th>
<th>16-cell</th>
<th>Blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 8.9. Oocyte and embryo production from donor cow No. 857 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH  
BCS: 6.0

Cow No. 2015

Date of calving: 02-01-2000 10 days postpartum

Oocyte Aspiration

<table>
<thead>
<tr>
<th>Ovary size</th>
<th>Follicles</th>
<th>Follicles aspirated</th>
<th>Oocytes recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>right: 4.0 cm</td>
<td>right: 15</td>
<td>right: 13</td>
<td>15</td>
</tr>
<tr>
<td>left: 3.0 cm</td>
<td>left: 15</td>
<td>left: 12</td>
<td></td>
</tr>
</tbody>
</table>

Oocyte recovery rate: 60%

<table>
<thead>
<tr>
<th>Oocyte Quality Grades</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 8</td>
<td>8</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Embryo Production

<table>
<thead>
<tr>
<th>n</th>
<th>2-cell</th>
<th>16-cell</th>
<th>Blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 8.10. Oocyte and embryo production from donor cow No. 2015 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH  
BCS: 6.0

Cow No. 779  

Date of calving: 03-12-2000  
15 days postpartum

Oocyte Aspiration

<table>
<thead>
<tr>
<th>Ovary size</th>
<th>Follicle</th>
<th>Follicle aspirated</th>
<th>Oocytes recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>right: 2.0 cm</td>
<td>right: 8</td>
<td>right: 7</td>
<td>12</td>
</tr>
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</table>

Oocyte recovery rate: 100%

<table>
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<tr>
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<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
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<tbody>
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<td>1</td>
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Embryo Production

<table>
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<th>Blastocysts</th>
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<tbody>
<tr>
<td>9</td>
<td>7</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 8.11. Oocyte and embryo production from donor cow No. 779 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH
BCS: 6.0

Cow No. 887

Date of calving: 03-27-2000 15 days postpartum

Oocyte Aspiration

<table>
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<tr>
<th>Ovary size</th>
<th>Follicles</th>
<th>Follicles aspirated</th>
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<tr>
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Oocyte recovery rate: 83%

Oocyte Quality Grades

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<th>D</th>
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Embryo Production

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<tbody>
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Figure 8.12. Oocyte and embryo production from donor cow No. 887 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH
BCS: 6.0

Cow No. 2074

Date of calving: 02-16-2000                       15 days postpartum

Oocyte Aspiration

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<th>Follicles</th>
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</thead>
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Oocyte recovery rate: 50%

Oocyte Quality Grades

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<th>C</th>
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Embryo Production

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<th>Blastocysts</th>
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</thead>
<tbody>
<tr>
<td>9</td>
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<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

Figure 8.13. Oocyte and embryo production from donor cow No. 2074 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH
BCS: 6.0

Cow No. 2182

Date of calving: 02-17-2000 15 days postpartum

Oocyte Aspiration

<table>
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<tr>
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Oocyte recovery rate: 100%

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Embryo Production

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</thead>
<tbody>
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Figure 8.14. Oocyte and embryo production from donor cow No. 2182 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH  
BCS: 6.0

Cow No. 7064

Date of calving: 02-14-2000  15 days postpartum

Oocyte Aspiration

<table>
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<tr>
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<th>Follicles</th>
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</tr>
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Oocyte recovery rate: 100%

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<tbody>
<tr>
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Embryo Production

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<tbody>
<tr>
<td>1</td>
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<td>1</td>
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</table>

Figure 8.15. Oocyte and embryo production from donor cow No. 7064 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH
BCS: 6.0

Cow No. 4029

Date of calving: 02-19-2000
20 days postpartum

Oocyte Aspiration

<table>
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<th>Follicles</th>
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Oocyte recovery rate: 75%

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Embryo Production

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</thead>
<tbody>
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<td>1</td>
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</table>

Figure 8.16. Oocyte and embryo production from donor cow No. 4029 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH  
BCS: 6.0

Cow No. 6025

Date of calving: 02-19-2000 20 days postpartum

<table>
<thead>
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<th>Follicles aspired</th>
<th>Oocytes recovered</th>
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Oocyte recovery rate: 62%

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<td>C</td>
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<tr>
<td>D</td>
<td>5</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
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<td>n</td>
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<tr>
<td>----</td>
</tr>
<tr>
<td>7</td>
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</tbody>
</table>

Figure 8.17. Oocyte and embryo production from donor cow No. 6025 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH
BCS: 5.0

Cow No. 6038

Date of calving: 02-01-2000                     20 days postpartum

Oocyte Aspiration

<table>
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<th>Ovary size</th>
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Oocyte recovery rate: 45%

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Embryo Production

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<tbody>
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Figure 8.18. Oocyte and embryo production from donor cow No. 6038 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH
BCS: 6.0

Cow No. 6622

Date of calving: 03-18-2000 20 days postpartum

Oocyte Aspiration

<table>
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<tr>
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Oocyte recovery rate: 60%

Oocyte Quality Grades

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Embryo Production

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<tbody>
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</table>

Figure 8.19. Oocyte and embryo production from donor cow No. 6622 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH
BCS: 6.0

Cow No. 5028

Date of calving: 03-21-2000                     20 days postpartum

<table>
<thead>
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<tr>
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Oocyte recovery rate: 80%

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</table>

Figure 8.20. Oocyte and embryo production from donor cow No. 5028 (Oocyte and embryo production from FSH-treated postpartum beef cows shortly after calving).
Treatment: FSH + PVP
BCS: 6.0

Cow No. 5514

Date of calving: 12-10-2000 10 days postpartum

Oocyte Aspiration

<table>
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<tr>
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>30 immature oocytes

Oocyte recovery rate: 76%

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Embryo Production

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<tbody>
<tr>
<td>11</td>
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<td>4</td>
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</table>

Figure 8.21. Oocyte and embryo production from donor cow No. 5514 (Effect of Somatotropin and single FSH-dose on oocyte production from postpartum beef cows 10 days after parturition).
Treatment: FSH

Cow No. 1615

Date of calving: 09-29-2000  25 days postpartum

Oocyte Aspiration

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<tbody>
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<tr>
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Oocyte recovery rate: 94%

Oocyte Quality Grades

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Embryo Production

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<tbody>
<tr>
<td>13</td>
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<td>2</td>
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Figure 8.22. Oocyte and embryo production from donor cow No. 1615 (Transvaginal ultrasound-guided oocyte aspiration from FSH-treated postpartum dairy cows.)
Treatment: FSH
BCS: 6.0

Cow No. 5524

Date of calving: 12-10-2000 10 days postpartum

Oocyte Aspiration

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<th>Oocytes recovered</th>
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</tr>
<tr>
<td>left: 3.0 cm</td>
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Oocyte recovery rate: 76%

<table>
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Embryo Production

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<th>Blastocysts</th>
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<tbody>
<tr>
<td>11</td>
<td>7</td>
<td>4</td>
<td>1</td>
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</tbody>
</table>

Figure 8.23. Oocyte and embryo production from donor cow No. 5524 (Effect of somatotropin and single FSH-dose on oocyte production from postpartum beef cows 10 days after parturition).
Figure 8.24. Pregnancies detected by ultrasonography at 60 days of gestation from embryos produced by cows during the first 20 days postpartum.
Figure 8.25. Pregnancies detected by ultrasonography at 60 days of gestation from embryos produced by cows during the first 20 days postpartum.
VITA

Oscar Perez was born on May 11, 1961, in Mexico City, Mexico, of parents Rosa Alcantara and Carlos Perez. Oscar is the oldest of six children. He graduated from the National School of Teachers in Mexico City in 1980. He taught in four Elementary Schools of Mexico City for 7 years. He attended the Metropolitan Autonomous University in Mexico City to pursue a degree in Veterinary Medicine in 1987. During this time he focused on bovine reproduction. In 1987, he began his master’s degree program in animal reproduction at the Autonomous University of Chapingo. During his master’s program, Oscar studied bovine embryo transfer technology. Before he finished his master’s degree, he began working as assistant professor in applied bovine reproduction at the University of Chapingo. He taught 10 bovine artificial insemination courses in many states of Mexico. At the beginning of 1991, he moved to South Mexico to work as assistant researcher in the Postgraduate College. He also participated in an embryo transfer program in the Mexican tropics with purebred beef breeders working with Nelore, Indubrasil, Simmental, Charolais and Brown Swiss cattle, collecting, transferring and freezing embryos for 2 years.

In May 1994, he visited the Saskatchewan University in Canada to attend an embryo transfer workshop with Dr. Rouben Mapleton. He then attended a Ph.D. program in animal reproduction in the United States but he had to wait 3 years to obtain his scholarship. During this time, Oscar moved to the central part of Mexico to work as assistant researcher at Postgraduate College. He served as veterinarian for the cattleman’s association of Salinas de Hidalgo S.L.P. from 1994 to 1997. In this place, he founded the serology laboratory with a national board accreditation in brucelosis and tuberculosis. He was responsible for monitoring brucelosis and tuberculosis in >18,000 cows. In August of 1987, he and his family traveled to the USA where he was accepted into the LSU Embryo Biotechnology Laboratory graduate program under the direction of Dr. Robert A. Godke of the Department of Animal Sciences at Louisiana State University, where he is currently a candidate for the Doctor of Philosophy degree in reproductive physiology.