

EFFECTS OF THE NUMBER OF COWS IN ESTRUS  
AND CONFINEMENT AREA ON ESTROUS  
BEHAVIOR OF BEEF COWS

By

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## CHAPTER I

### INTRODUCTION

In 2000, there were over 98 million cattle and calves in the United States with a total value of over 67 billion dollars, 5.2 million of these cattle were located in Oklahoma (USDA, 2001b). The importance of the cattle industry cannot be underestimated, with cattle and calves providing 36.8 and 2.1 billion dollars of gross income in the US and Oklahoma, respectively, in 2000 (USDA, 2001b). Beef production totaled a record 26.8 billion pounds in 2000 and the increasing demand for higher quality beef resulted in the highest retail prices for choice beef since 1993 (USDA, 2001a). Consumers are driving the market for higher quality and consistency in beef and per capita beef consumption has tended to increase since 1997 (USDA, 2001a).

Artificial Insemination (AI) allows the introduction of new and superior genetics to an increased number of cows than with natural service. Superior and more consistent genetics will help improve the quality and consistency of beef. However, AI has not been widely implemented throughout the beef industry. In 1997, only 7.1% of beef operations used AI (USDA, 1998). Of those implementing AI, 37% are operations with 300 or more cows (USDA, 1997). Two major factors cited for not using AI are the time and labor involved (38.8%) and complications (19.6%) that it introduces (USDA, 1997).

A prerequisite for successful AI is insemination at the correct time relative to ovulation. The most definitive external sign of approaching ovulation is estrous



behavior. In dairy cows, the greatest conception rates occur when cows were inseminated 4 to 12 h after the onset of estrus (Dransfield, 1998). Sixty-three percent of beef heifers and suckled beef cows conceived, when insemination at 12 to 16 h after detected estrus (Stevenson et al., 1997). Ovulation occurs about 31 h after the onset of estrus in beef cows (White et al., 2002).

Estrous behavior has been widely studied in dairy cows but limited research has been conducted with beef cows. Accurate detection of estrus can be made by visual observations. Several observations throughout the day for 30 min to an hour detected 77 to 90 % (Donaldson, 1968; Stevenson et al., 1996b) of cows in estrus, but efficiency of estrous detection increases when the number of observation periods increase (Hall et al., 1959). Twenty-seven percent of estruses in beef heifers were not detected with twice daily visual observation (Stevenson, 1996b).

Many factors effect the expression of estrous behavior in cattle. A better understanding of factors that can be easily changed by producers to increase the number of cows detected in estrus could decrease labor involved and facilitate the use of AI in the beef industry. Confinement area might influence estrous behavior of cattle (Mattoni, 1988). The number of mounts and duration of estrus increases as the number of dairy cows in estrus at one time increases (Hurnik et al., 1975; Helmer and Britt, 1985; Walton et al., 1987; Van Vliet and Van Eerdenburg, 1996). Further investigation of how these factors influence estrous behavior is warranted.

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## CHAPTER II

### REVIEW OF LITERATURE

#### Introduction

Estrus is only a short period in the cycle of hormonal changes that occur during the 21 d bovine estrous cycle. The cycle is composed of the luteal phase and the follicular phase. The luteal phase begins after ovulation when the corpus luteum (CL) forms, and is characterized by growth of the CL and production of progesterone. The follicular phase, which is predominated by preovulatory follicle growth and production of estradiol, begins after CL regression and ends at ovulation. Complex interactions between the hypothalamus, pituitary, ovary and uterus regulate reproductive processes and will be discussed in this section.

Hormonal factors that regulate the estrous cycle culminate in the expression of estrus or standing heat. During estrus, cows become interested in mating with other cattle, and actively seek mounting partners and bulls inseminate cows. Numerous behavioral changes are associated with estrus, but the most consistent and accurate visual sign that a cow is in estrus is for the cow to stand and remain immobile for another cow or bull to mount (Esslemont et al., 1980). The behavioral expression of estrus is influenced by complex social, environmental, and animal interactions.

### Endocrine Control of the Estrous Cycle

During the luteal phase of the estrous cycle, concentrations of progesterone in plasma increase ( $P < 0.05$ ) from d 1 (d 0 = estrus) through d 17 (Swanson et al., 1972; Ireland et al., 1979) due to growth of the CL and increased production of progesterone. Increased concentrations of progesterone in plasma have a negative feedback on the hypothalamus and decrease release of gonadatropin releasing hormone (GnRH). Infrequent and minimal pulses of GnRH result in infrequent pulses of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary. Most cattle have 2 to 3 waves of follicular growth during each cycle (Sirois and Fortune, 1988; Ginther et al., 1989; Melvin et al., 1999). Variation in number of follicular waves in each estrous cycle accounts for some of the variation in estrous cycle length. Waves of follicular growth consist of three phases: recruitment, selection, and dominance. An increase in FSH stimulates the first wave of follicular growth (Adams et al., 1992). During recruitment, a group of follicles from the egg nest begin to grow and produce estradiol. In the selection phase, some follicles become atretic and the others continue to grow and keep produce estradiol. One of the follicles from the selection phase will become dominant over the others, through possible interaction of insulin-like growth factor binding protein-4 and estradiol (Mihm et al., 2000). Maximum concentrations of progesterone in plasma during the luteal phase inhibit ovulation of dominant follicles, which become atretic and regress. Minimal ( $< 1$  ng/ml) concentrations of progesterone during the follicular phase are permissive for ovulation.

Reduced progesterone concentrations between the luteal and follicular phases reflect regression and demise of the CL. The CL produces oxytocin during the later stage of the cycle in response to minimal concentrations of Prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) produced by the uterus. Oxytocin acts on the uterine epithelium during the late luteal phase to cause release of  $PGF_{2\alpha}$ . Uterine  $PGF_{2\alpha}$  is transported to the CL by means of counter current exchange in the utero-ovarian vein and artery and causes luteolysis of the CL and reduced production of progesterone approximately 3 d before estrus. Concentrations of progesterone in plasma decrease from 4 to 8 ng/mL during the late luteal phase, to less than 1 ng/mL within 48 h of natural luteolysis (Henricks et al., 1970; Swanson et al., 1972; Wettemann et al., 1972), are minimal at estrus (Ireland et al., 1979; Glencross et al., 1981) and remain minimal until 2 d after estrus (Garverick et al., 1971). Minimal concentrations of progesterone alleviate the negative feedback of progesterone on the hypothalamus and allow increased pulsatile secretion of GnRH which results in increased pulsatile release of FSH and LH. Increased secretion of FSH and LH stimulate follicular growth and production of estradiol. Lutenizing hormone binds to membrane receptors on the surface of thecal cells of growing follicles and causes production of androstendione, which diffuses across the basement membrane into granulosa cells (Hansel and Convey, 1983). Follicle stimulating hormone binds to membrane receptors on granulosa cells causing an increase in aromatase activity, which converts androstendione to estradiol 17- $\beta$  (Hansel and Convey, 1983). Concentrations of estradiol increase from approximately 2 pg/mL at 4 d before estrus to 5 to 10 pg/mL around the start of standing estrus (Wettemann et al., 1972; Echterkamp and Hansel, 1973; Chenault et al., 1975). Concentration of estradiol in plasma decrease by 12 h after the onset of estrus (Glencross et al., 1981) or about 8 h before ovulation (Chenault et al.,

1975; White et al., 2002). Estrous behavior is stimulated by increased concentration of estradiol in the relative absence of progesterone (Hurnik, 1987). Increased concentrations of estradiol stimulate the GnRH pulse generator in the hypothalamus resulting in the preovulatory surge of GnRH and LH. The dominant follicle secretes inhibin which inhibits a preovulatory surge of FSH that would otherwise be caused by the preovulatory surge of GnRH (Kaneko et al., 1997). The ovulatory surge of LH occurs shortly after maximum concentrations of estradiol in plasma (Walters and Schallenberger, 1984; Kaneko et al., 1991) and is highly correlated with the onset of estrus ( $P < 0.05$ ,  $R = 0.962$ ; Rodtian et al., 1996). The preovulatory surge of LH occurs 2 to 14 h after the onset of standing estrus (Henricks et al., 1970; Chenault et al., 1975; Larsson, 1987) and persists for 8 to 12 h (Swanson and Hafs, 1971; Christensen et al., 1974). Ovulation occurs 24 h (Christensen et al., 1974; Larsson, 1987) after the LH peak or about 31 h (Christensen et al., 1975; Larsson, 1987; Swanson and Hafs, 1971; White et al., 2002) after the onset of estrus. Cells of the follicle that remain after ovulation luteinize and develop into a CL that produces progesterone.

#### Hormonal Induction of Estrus

Estrous behavior can be induced in ovariectomized heifers and cows with the administration of hormones such as estradiol (Ray, 1965; Vailes et al., 1992) or testosterone (Nessan and King, 1981). Estradiol benzoate (EB), given at 600, 1200, 2400, and 4800  $\mu\text{g}$  to ovariectomized Holstein cows and heifers induced estrus in 90 to 100 percent of animals, which was greater ( $P < 0.05$ ) than the response to a 300  $\mu\text{g}$  dose (40%; Cook et al., 1986). The interval from treatment with EB to estrus was not different among doses ( $13.6 \pm 1.5$  to  $16.8 \pm 1.0$  h) and the duration of estrus was longer ( $P < 0.05$ )

only with the 4800 mg treatment ( $20.2 \pm 3.1$  vs  $9.6 \pm 1.4$  to  $13.0 \pm 1.9$  h). The minimal amount of EB to induce estrus in a majority of ovariectomized cows was 500  $\mu$ g (Cook et al., 1986). Ovariectomized cows that were primed with progesterone before EB treatment exhibited similar estrous behavior to those that were treated with only EB, while estrous behavior was suppressed in cows that were given EB when plasma concentrations of progesterone were similar to the luteal phase (Vailes et al., 1992). There was a linear decrease ( $P < 0.01$ ) in the percentage of ovariectomized Holstein cows with estrous behavior and a linear increase ( $P < 0.02$ ) in the interval to estrus after treatment with EB as cows were treated with greater concentration of progesterone (200, 600, or 1000 mg progesterone/d for 5 d beginning 3d prior to EB; Davidge et al., 1987). Estrous behavior induced in ovariectomized cows with EB (0.5 mg) was not influenced by progesterone (10 mg), GnRH (0.4 mg) or testosterone propionate (12.5 mg), while treatment with dexamethasone (4 mg; Cook et al., 1987) decreased ( $P < 0.01$ ) the number of cows exhibiting estrus but did not influence the behavioral characteristics of those that exhibited estrus (Allrich et al., 1989). Cook et al., (1986) found that GnRH did not influence estrous expression when given with low doses of EB. In addition treatment with cortisol did not affect estrous response in ovariectomized dairy cows given EB (Cook et al., 1987). The estrous response was different in Hereford, Brahman and crossbred cows when treated with estradiol (Rhodes and Randel, 1978). Testosterone will induce estrous behavior when given to intact animals in large amounts (100 to 400 mg; Allrich, 1994; Kiser et al., 1977), but results are not as conclusive when given to ovariectomized cows (Nessan and King, 1981).

## Synchronization of Estrus

There are four basic hormones used in estrous synchronization: PGF<sub>2α</sub>, estrogen, progestogens (progesterone and MGA), and GnRH. Prostaglandin F<sub>2α</sub> and progestogens can be used alone or in combination with estrogen and GnRH. Of these compounds, PGF<sub>2α</sub> is currently used most extensively in the U.S. and was used in the experiment reported in this thesis.

### Prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>)

Prostaglandin F<sub>2α</sub>, and analogs of PGF<sub>2α</sub>, cause luteolysis of the CL similar to endogenous PGF<sub>2α</sub> produced by the uterine epithelium. Concentrations of progesterone in plasma decrease to less than 1ng/ml within 24 h after treatment with PGF<sub>2α</sub> (Louis et al., 1974; Chenault et al., 1976). The decrease in progesterone secretion is followed by increased secretion of estradiol and LH, similar to changes during natural luteolysis. Time of ovulation ( $27.6 \pm 5.4$  h) did not differ between estruses induced by PGF<sub>2α</sub> and spontaneous estruses (Walker et al., 1996). Duration of estrus and mounts per estrus were similar during normal and induced estruses (Stevenson et al., 1996a; Walton et al., 1987) which agrees endocrine secretions after normal and induced estruses (Glencross and Pope, 1981).

Prostaglandin F<sub>2α</sub> does not cause luteolysis when given before d 5 of the estrous cycle (See Lauderdale and Sokoloski, 1979 for review). Thus, a synthronization method was developed in which two injections are given 10 to 14 d apart. If cattle are equally distributed among days of the estrous cycle, approximately 70% should respond to the first injection. Those not responding to the first injection (cows on d 18 to d 4 of the

cycle) should respond to the second injection. Cows that respond to the first treatment with PGF<sub>2α</sub> will be at d 7 to 9 of the next estrous cycle at the second treatment and should respond. Even with this treatment regimen not all animals exhibit estrus (Table 1).

Increasing the interval between two PGF<sub>2α</sub> treatments from 11 d to 14 d prolonged the luteal phase of the 14 d group which allowed for greater CL growth and greater progesterone concentrations at the time of PGF<sub>2α</sub> administration (Rosenberg et al., 1990).

When PGF<sub>2α</sub> treatments were given 11 d apart, the interval to estrus following the second injection was shorter ( $P < 0.05$ ) than the interval to estrus after the first treatment, and similar to when a single injection was given on d 8 of the estrous cycle (Johnson, 1978). The interval between PGF<sub>2α</sub> treatment and estrus was not affected by breed (King et al., 1982) or the number of cows in estrus at the same time (Walton et al., 1987).

Treatment with PGF<sub>2α</sub> does not influence fertility, although Morrell et al., (1991) suggested that fertility was decreased after several successive treatments with PGF<sub>2α</sub>. Pregnancy rates after AI of beef cows (suckled and non-suckled), dairy cows, and beef and dairy heifers treated with PGF<sub>2α</sub> were not different than for control animals AI following natural estrus (Lauderdale et al., 1974; Lauderdale and Sokoloski, 1979; Jackson et al., 1983). Dairy heifers treated with PGF<sub>2α</sub> on d 5 to 7 had reduced ( $P < 0.05$ ) first service conception rate (56.8%) compared with those treated on d 12 to 15 (78.3%; Watts and Fuquay, 1985).

Day of the estrous cycle at which PGF<sub>2α</sub> treatment is administered affects the interval to onset of estrus and the synchrony of estrus. Heifers treated on d 5 to 9 exhibited estrus 12 h earlier ( $P < 0.01$ ) than heifers treated on d 10 to 15 (King et al., 1982). Dairy cows treated in mid-diestrus (d 12 to 14) had longer intervals to estrus after



Table 1. Summary of selected references on the use of PGF<sub>2α</sub> in cattle

Animal Type	Treatment	Interval to estrus, h	Percent response, %	Reference
Dairy cows	10 mg in uterine horn or 30 mg i.m. on d 8-13 of the cycle	74.9 ± 21.1	NA	Chenault et al., 1976
Holstein heifers	2 PGF <sub>2α</sub> 12 d apart (2 <sup>nd</sup> given at 0600 or 1800)	69.8 ± 4.4 72.7 ± 5.2	76 81	Britt et al., 1978
Hereford/Freisian heifers	1 <sup>st</sup> PGF <sub>2α</sub> 2 <sup>nd</sup> PGF <sub>2α</sub> 11 d later PGF <sub>2α</sub> on d 8	68.6 ± 20.8 59.9 ± 15.8 57.4 ± 12.5	25 41 43	Johnson, 1978
Beef cows (suckled)	2 PGF <sub>2α</sub> 11 d apart	NA	47 ± 6	Lauderdale and Sokoloski, 1979
Beef cows (non-suckled)		NA	80 ± 10	
Beef heifers		NA	66 ± 4	
Dairy heifers		NA	73 ± 21	
Angus, Hereford, and Simmental heifers	PGF <sub>2α</sub> on d 0 and d 11 or at d 5 to 8 or d 12 to 15	53.6 ± 1.0	NA	King et al., 1982
Angus and Hereford cows		61.9 ± 1.1	NA	
Holstein heifers	PGF <sub>2α</sub> on d 5 to 8 d 14 to 16	49.5 ± .6 60.6 ± .8	84 83	Stevenson et al., 1984
Holstein heifers	PGF <sub>2α</sub> on d 7 d 11 d 15	43.9 ± 8.2 h 71.5 ± 14.3 53.0 ± 12.2	86.0 90.0 98.0	Tanabe and Hann, 1984
Hereford cows & heifers	2 PGF <sub>2α</sub> 11 d apart (2 <sup>nd</sup> given at 0600 or 1800 h)	62.8 ± 5.2 57.6 ± 4.8	71 83	Nkuuhe and Manns, 1985
Dairy heifers	PGF <sub>2α</sub> after observed estrus on d 5-7 (n = 86) d 8-11 (n = 104) d 12-15 (n = 60)	59.3 ± 5.0 70.5 ± 2.2 72.0 ± 2.1	43.0 83.6 100	Watts and Fuquay, 1985
Holstein Cows	PGF <sub>2α</sub> upon identification of a CL via rectal palpation	73.1 ± 2.8	NA	Walker et al., 1996
Dairy cows	PGF <sub>2α</sub> every 11 d for 5 to 6 times	54.8 ± 16.6	68	Castellanos et al., 1997
Dairy heifers		57.0 ± 11.5	74	

NA – Data not available.

PGF<sub>2α</sub> than those that were treated in early and late diestrus (Macmillan and Henderson, 1984). Intervals to estrus were shortest for heifers treated with PGF<sub>2α</sub> on d 7 (43.9 ± 8.2 h), and longest for heifers treated on d 11 of the estrous cycle (71.5 ± 14.3 h; Tanabe and Hann, 1984). Another group of dairy heifers treated over a greater range of days had a shorter interval to estrus on d 5 to 7 (59.3 ± 5.0 h) than those treated on d 8 to 11 (70.5 ± 2.2 h) or 12 to 15 (72.0 ± 2.1 h; Watts and Fuquay, 1985). Stevenson et al., (1984) found that the interval to estrus in Holstein heifers treated with PGF<sub>2α</sub> on d 5 to 8 (49.5 h) was shorter than for heifers treated on d 14 to 16 (60.6 h). Fewer dairy heifers treated on d 5 to 7 (43.0%) responded to PGF<sub>2α</sub> in the 5 d following treatment than those treated on d 8 to 11 (83.6 %) or 12 to 15 (100%; Watts and Fuquay, 1985). However, Tanabe and Hann, (1984) and King et al., (1982) found no differences in the percentage of animals exhibiting estrus when dairy heifers or beef cows and heifers were treated with PGF<sub>2α</sub> on different days of the cycle. Stage of the cycle at treatment affects the synchrony of estrus (Refsal and Seguin, 1980). More dairy heifers treated with PGF<sub>2α</sub> on d 7 and 15 of the cycle exhibited estrus in a 24 h period than those treated on d 11 of the cycle, however day of treatment did not influence fertility (Tanabe and Hann, 1984).

Intervals from treatment with PGF<sub>2α</sub> to estrus (Britt et al., 1978; Walton et al., 1987), the ovulatory surge of LH, and ovulation (Nkuuhe and Manns, 1985) were similar when the second of two PGF<sub>2α</sub> treatments was given 11 d after the first at either 0600 or 1800 h. Time of day that PGF<sub>2α</sub> treatment was administered did not affect fertility (Britt et al., 1978).

## Estrogen

Estrogen is luteolytic early in the estrous cycle (Wiltbank, 1966). While it is not used by itself for estrous synchronization, it is often used in conjunction with a progestogen. When given at the beginning of progesterone treatment, estrogen will induce a new wave of follicular growth regardless of the stage of the dominate follicle at the time of treatment, and therefore decreases the chance of persistent dominant follicles (Bo et al., 1994).

## Progestins

Progestin treatment can be given via injection (Christian and Casida, 1948), incorporation into feed (melengestrol acetate – MGA (Zimbelman and Smith, 1966) or by a device that is inserted into the vagina and has a slow release over time (controlled intravaginal drug release device – CIDR; Macmillan and Peterson, 1993) or progesterone releasing internal device – PRID; Munro and Moore, 1985; Smith et al., 1984). Treatment is given to maintain adequate concentrations of progestin and inhibit ovulation for an extended period of time. Progesterone inhibits estrus (Christian and Casida, 1948; Davidge et al., 1987) and suppresses LH release. This prevents ovulation and causes synchronization of estrus when treatment is ended. While the surge of LH is suppressed, LH pulse frequency may increase (Stock and Fortune, 1993). Prolonged treatment with progesterone causes synchrony of estrus but reduced fertility at estrus due to the development of persistent follicles propagated by the high LH pulse frequency (Stock and Fortune, 1993). Persistent follicles can be avoided by causing atresia of the dominant follicle at the beginning of progesterone administration. This can be accomplished by pre-treatment with estrogen or GnRH. Progesterone in combination with estrogens will

induce cyclicity in anestrous cows (Macmillan and Burke, 1996; Lammoglia et al., 1998) and heifers (Macmillan and Peterson, 1993; Rasby et al., 1998).

Prostaglandin  $F_{2\alpha}$  can be given at the end of short term progesterone treatment (Smith et al., 1984; Kesler et al., 1996; Lucy et al., 2001). Holstein heifers treated with progesterone (PRID) for 6 d and given  $PGF_{2\alpha}$  at PRID removal had similar pregnancy rates (72 to 82%) but less synchrony of the onset of estrus than heifers treated with progesterone (PRID) for 7 d and  $PGF_{2\alpha}$  given one day before PRID removal (Smith et al., 1984). The latter treatment also induced estrus in a greater percentage of heifers and heifers had greater pregnancy rates than heifers treated with two  $PGF_{2\alpha}$  injections 11 d apart. Progesterone treatment for 7 d with  $PGF_{2\alpha}$  given the day before removal improved synchronization and pregnancy rates for beef cows and heifers compared with one treatment with  $PGF_{2\alpha}$  (Lucy et al., 2001).

Treatment with progestogen (3 mg of norgestomet at insertion of a 6-mg norgestomet ear implant) for 9 d in combination with estradiol valerate (EV; 5 mg at implant insertion), previously commercially available as Syncro-Mate B, yielded an estrus response of 77 to 100 % with first service conception rates from 33 to 68% (see Odde, 1990 for review). Syncro-Mate B produced adequate synchrony of estrus and ovulation, and day of the estrous cycle that treatment was initiated did not affect conception rate or the interval from removal to ovulation (Mikeska and Williams, 1988). Similar treatment with EB at the onset of a 7 d progesterone treatment (CIDR), with  $PGF_{2\alpha}$  and EB given at removal, resulted in synchronized estrus in 98% of cyclic beef heifers and a conception rate of 62.0 % during 4 d, which was similar to conception rate in unsynchronized heifers (Lemaster et al., 1999). Estradiol benzoate given 24 to 30 h

after seven day progesterone treatment (CIDR), with PGF<sub>2α</sub> given the day before removal, increased expression of estrus and degree of synchrony in beef heifers compared with heifers given a progesterone (CIDR) for 7 d with PGF<sub>2α</sub> administered the day before removal (Lammoglia et al., 1998). This and other evidence (Johnson et al., 1997) suggest that EB treatment at the end of progestogen treatment increases the synchrony of estrus. Prostaglandin F<sub>2α</sub> given at the end of short term progesterone treatment resulted in a similar percentage of animals exhibiting estrus and conception rates as when estradiol-17β or GnRH was incorporated at the beginning of progesterone treatment (Martinez et al., 2000).

#### Gonadotropin Releasing Hormone (GnRH)

Administration of GnRH or GnRH analogs at different stages of the estrous cycle causes a surge of LH and ovulation. This will initiate the emergence of a new follicular wave within 3 to 4 d (Twagiramungu et al., 1995; Kohram et al., 1998). GnRH treatment is used in three popular protocols; Ov-Synch, CO-Synch and Select Synch (see Whittier and Geary, 2000, for review). The first injection (d 0) will cause turnover of the follicular wave and a new CL to form. This is followed by treatment with PGF<sub>2α</sub> (d 7) to cause luteolysis and maturation and ovulation of the dominant follicle. With the Select Synch protocol, cows are inseminated at detected estrus. Ov-synch and CO-Synch require another injection of GnRH on d 9 to cause ovulation and CO-Synch cows would be inseminated at that time. Cows on the Ov-Synch protocol would be inseminated on d 10. GnRH produces a greater synchronization of ovulation with the majority of cows ovulating in an 8 h period (24 to 32 h after treatment; Pursley et al., 1995). While some

(Kojima et al., 2000; Stevenson et al., 2000) suggest that GnRH protocols induce cyclicity in anestrus cows, it is not effective in thin anestrus beef cows (Looper et al., 2000).

### Estrous Behavior in Cattle

Many physiological changes occur during estrus that can be detected either visually or with the aid of technology. Characteristics that occur during most estruses, and are easily observed, are known as primary characteristics. Others, described as secondary characteristics, may occur with every estrous period but are more difficult to observe.

#### Primary Characteristics

Numerous physiological and behavioral changes are associated with estrus and ovulation, but the most consistent and accurate visual sign of estrus is for a cow to stand and remain immobile for another female or male to mount (Hurnik et al., 1975; Esslemont et al., 1980). The duration of estrus is defined as the interval from the first time a cow stands to be mounted until the last mount. This is also known as standing estrus. Both the number of mounts and the duration can vary greatly from 6 to 60 mounts per estrus and 4 to 18 h in duration (Table 2). Before and after standing estrus, other cows may attempt to mount the cow, but she will move away. This is called an "attempted mount". Holstein heifers maintained in groups of four would continually attempt to mount an estrual heifer before ( $5.4 \pm 5.8$  h) and after ( $2.3 \pm 3.9$  h) standing estrus occurred (Hurley et al., 1982). Cows mounting other cows can be an indication of proestrus, estrus or metestrus. Ninety percent of cows that received mounts were in estrus, whereas 70% of cows were estrus that were mounting others (Hurnik et al., 1975).

Table 2. Estrous behavior of beef and dairy cows determined by different methods of detection

Type	Description	Method of detection	Duration of standing estrus, h	Mounds/estrus, no	Reference
Dairy Cow	Lactating	HeatWatch®	7 - 10,	8 - 11	Dransfield et al., 1998; Walker et al., 1996; Xu et al., 1998
Dairy Cow	Lactating	Continuous observation	8 - 10	7 - 33	Pennington et al., 1985; Walton et al., 1987
Dairy Heifer	Synchronized	Continuous observation	15	60	Hurley et al., 1982
Beef Cow	Postpartum	HeatWatch®	4 - 6	6 - 13	Ciccioli and Wettemann, 2000; Lents 2001
Beef Cow	Postpartum	Continuous observation	4.4		Hurnik and King, 1987
Beef Cow	Non-suckled	Continuous observation	12 - 15	18 - 41	Galina et al., 1982; Orihuela et al., 1983
Beef Cow	Non-suckled	HeatWatch®	14 - 18	38 - 59	White et al., 2002
Beef Heifer	Synchronized	HeatWatch®	8 - 9	27 - 50	Rae et al., 1999; Stevenson et al., 1996b

During 30 min daily visual observation periods of Zebu cows, 87.9% of all attempted mounts and mounts were performed by cows in estrus (Vaca et al., 1985). Nonestrus cows frequently mount estrus cows (Williamson et al., 1972a). Estrus intensity and duration did not affect conception rates in lactating dairy cows inseminated on a once a day schedule (Dransfield et al., 1998). Some cows experience ovulation without estrous behavior, sometimes called silent ovulation. Ovulations may occur 9 to 26 % of the time without estrus (Plasse et al., 1970; Orihuela et al., 1983; Rodtian et al., 1996).

### Secondary Characteristics

Although standing to be mounted is the only clear-cut sign of estrus, the frequency of other activities change near estrus as well. The amount of time during the day spent standing increased 32%, ambulation increased 40%, and lying down decreased 15% in confined beef cows on the day of estrus when compared ( $P < 0.01$ ) with nonestrus days (Hurnik and King, 1987). Similar changes in activity occurred in dairy cows that grazed all or part of the day or were maintained in a straw yard (Phillips and Schofield, 1990). Ambulation increased by 100 % to 393 % during estrus (Kiddy, 1976; Lewis and Newman, 1984; Pennington et al., 1986; Redden et al., 1993).

Investigative (sniffing, rubbing, licking, and chin resting) and aggressive behavior (butting) increased before, during and after estrus but are not confined to these periods (Esslemont et al., 1980). These secondary characteristics of estrous behavior begin about 12 h before standing estrus, increase in frequency until standing estrus, and decrease in the 12 h after estrus (Allrich, 1993). In Holstein cows maintained in a free stall area and observed with a timelapse video, the frequency of fights and/or threats approximately



doubled during estrus (Hurnik et al., 1975). Phillips and Schofield, (1990) concluded that aggression may be confined to estrus when animals are maintained in a larger area, but when they are in more confined spaces, aggression can be displayed in nonestrus animals. When contrasting the day of estrus to the two days before or after, there was an increase ( $P < 0.001$ ) in chin pressing, licking, and sniffing in primiparous dairy cows while only an increase ( $P < 0.01$ ) in chin pressing and licking occurred in multiparous dairy cows (Amyot and Hurnik, 1987). Confined beef cows with suckling calves initiated and received more sniffs, licks and chin presses around estrus than when not near estrus (Hurnik and King, 1987).

Dynamic physiological changes in the female reproductive tract occur near estrus. Clear vaginal mucus is expelled through the vulva and is often seen on the hindquarters or tail. Mucus discharge occurred in 64% of estruses in Zebu cattle under continuous observation (Mattoni et al., 1988) and 68% of dairy cows identified by testosterone treated females with chin-ball markers (Hackett and McAllister, 1984). In a study of 732 dairy cows and heifers, vaginal mucus was present 50% of the time at AI after detected estrus, and conception rate was greater when mucus was present at breeding (48 vs 39% conception; Stevenson et al., 1983). Vaginal temperature increases about 0.6 to 0.9 °C for about 7 h during estrus (Redden et al., 1993; Kyle et al., 1998). Time of maximal vaginal temperature was correlated with the time of the LH peak ( $r = 0.83$ ;  $P < .05$ ) and time of ovulation ( $r = 0.74$ ) (Rajamahendran et al., 1989). Lewis and Newman, (1984) found variation in vaginal temperature throughout the estrous cycle, but ambient temperature accounted for more of the variance than did day of the cycle. Vaginal pH was lowest on the day of estrus (Schilling and Zust, 1968; Lewis and Newman, 1984). There was a decrease in electrical resistance of vulvar tissue at estrus (Lewis et al., 1989)

due to the decreased cell density and increased extracellular and intracellular water (Ezov et al., 1990) which are regulated by progesterone and estrogen (Lewis et al., 1989).

A highly instable, low molecular weight, polar pheromone has been isolated from bovine urine collected at estrus (Dehnhard and Claus, 1996). Vaginal mucus from estrus cows has been used to induce mounting behavior in the same animal during diestrus and this response may be due to the pheromones in the mucus (Nishimura et al., 1991). An estrous related odor is present in excretions from the vulva and vestibule, vaginal fluid, urine, milk and blood plasma of cows during estrus (Kiddy, 1984).

#### Factors that Influence Estrous Behavior

Most factors that cause stress to cows can affect estrous behavior during both the short term and long term. There are many physical, environmental, animal and social factors that effect estrous behavior, which are not fully understood due to the complex interactions between them. Stress usually causes a release of ACTH which can inhibit estrus and ovulation (Hein and Allrich, 1992; Stoebel and Moberg, 1982).

#### Animal Factors

Older beef cows (5 to 6 yr) were mounted more times than younger cows (2 to 4 yr; Mathew et al., 1999). Primiparous dairy cows were estrus for almost 50% less ( $P < 0.05$ ) time than multiparous cows ( $7.4 \pm 1.4$  h and  $13.6 \pm 2.0$ h, respectively; Walker et al., 1996). The percentage of dairy cows detected in estrus with twice daily visual observation for 30 minutes increased (Peter and Bosu, 1986) and the duration of estrus increased (Pollock and Hurnik, 1979) as days postpartum increased. However, standing and mounting activity were not affected by parity or days postpartum in dairy cows when

testosterone treated animals were included for estrous detection (Hackett and McAllister, 1984). Dairy cows in thin body condition had a decreased duration of estrus compared with cows in good body condition when subjected to heat stress (Wolfenson et al., 1988), suggesting that body condition influences estrous behavior when cows are in poor condition.

### Physical Facilities and Handling

Area of pens and type of footing surface can influence estrous behavior. Estrous behavior is reduced when dairy cows are maintained on concrete surfaces compared with dirt or straw bedding (Vailes and Britt, 1990; Rodtian et al., 1996). Eighty percent of mounting activities occurred in the location of best footing and least crowding in the winter and summer (Pennington et al., 1985). Dairy cows and heifers housed in a barn had more mounts (8.7 mounts/h) than counterparts in a drylot (6.1 mounts/h) or pasture (5.5 mounts/h; Gwazdauskas et al., 1983). Zebu cows in a small drylot that were fed hay at night, and allowed to graze on pasture during the day exhibited more mounts in the drylot than when on pasture, as well as an increase in mounting when animals were gathered into a pen (Mattoni et al., 1988). A similar increase in mounting activity occurred when dairy cows were moved between paddocks and the milking parlor (Williamson et al., 1972b).

### Environment

Effects of season on estrous behavior can be caused by temperature, precipitation and/or photoperiod. Animals are stressed whenever the temperatures deviate from the animal's thermal neutral zone and they experience cold stress or heat stress. The degree

to which season effects estrous behavior is somewhat controversial. Trimberger (1948) found that season had no effect on estrous behavior. Most studies demonstrate that season influences estrous behavior probably through complex interaction of duration and severity of temperature confounded with other factors that influence estrus. The duration and severity of temperature is largely a factor of geographical location. Heat stress reduces the number of mounts per estrus (Gangwar et al., 1965; Pennington et al., 1985; Nebel et al., 1997; White et al., 2002) but its influence on the duration of estrus is controversial. Duration of estrus was decreased when dairy heifers were exposed to artificial chronic heat treatment (33.5 C; Abilay et al., 1975) or artificial hot climatic conditions (Gangwar et al., 1965). However, under environmental heat stress the duration of estrus was longer in dairy cows (Pennington et al., 1985) and beef cows (Mattoni et al., 1988; Yelich et al., 1999; White et al., 2002). Geographical location may account for variation in the influence of heat stress on estrous behavior. Heat stress can cause anestrus in beef cows but cows adapted and resumed cyclity after a period of time (Bond and McDowell, 1972). Cooling and shading systems during hot weather prevented the detrimental effects of heat stress on estrous behavior (Wolfenson et al., 1988; Badinga et al., 1994). Cold stress decreased the number of estrous periods per month in *Bos indicus* cattle and was associated with little or no estrual activity, yet ovulation occurred (Plasse et al., 1970).

#### Time of Day

Effects of time of day on estrous behavior are not well established. Feeding, milking and moving cows at specific times of the day may influence behavior. Onset of estrus (Hurley et al., 1982; Rzepkowski et al., 1982; Walton et al., 1987; Xu et al., 1998)

and estrous behavior (Castellanos et al., 1997) may not be influenced by the time of day. However, Lamothe-Zavaleta et al., (1991) and Mattoni et al., (1988) found that 60% or greater of estruses began during the daylight in Zebu cows. Mounting behavior may be increased in the morning (De Silva et al., 1981; Galina et al., 1982; Gwazdauskas et al., 1983) or during dark (Esslemont and Bryant, 1976; Hall et al., 1959; Hurley et al., 1982; Hurnik et al., 1975; Williamson et al., 1972b). Esslemont and Bryant, (1976) and Hurnik et al., (1975) suggested that the increase in sexual activity during the night in dairy cows was caused by the absence of milking and feeding. Mounting activity decreased at the time of handling and feeding (Pennington et al., 1986). This may not necessarily be the case in beef cows since they are not gathered for milking and, depending on management practices and the season of the year, are probably not gathered on a daily basis for feeding. Variation in mounting activity associated with the light dark cycle could be influenced by seasonal variation. Dairy cows were mounted more in the warmest part of the day in the winter, and mounting was decreased during the hottest part of the day in the summer (Pennington et al., 1985).

### Social Factors

Social factors, such as, group size, social hierarchy and interaction, number of estrous females and the presence of a bull can influence estrous behavior. Cows in proestrus, estrus and metestrus tend to segregate from the main herd and form a sexually active group (Williamson et al., 1972b; Refsal and Seguin, 1980). Sexually active groups are more active than the rest of the herd, tend to stand closer together than other cows and remain together in pastures while most cows dissociate to graze (Williamson et al., 1972b). Synchronized cows and heifers interacted more often in the sexually active

UNLAFUWIR 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022 2023 2024 2025 2026 2027 2028 2029 2030 2031 2032 2033 2034 2035 2036 2037 2038 2039 2040 2041 2042 2043 2044 2045 2046 2047 2048 2049 2050 2051 2052 2053 2054 2055 2056 2057 2058 2059 2060 2061 2062 2063 2064 2065 2066 2067 2068 2069 2070 2071 2072 2073 2074 2075 2076 2077 2078 2079 2080 2081 2082 2083 2084 2085 2086 2087 2088 2089 2090 2091 2092 2093 2094 2095 2096 2097 2098 2099 2100

group (Castellanos et al., 1997). Galina et al., (1982) and Refsal and Seguin, (1980) found that dominant cows tend to mount other cows often, while they allow only a few cows to mount. Submissive cows mount a few cows, a limited number of times, while they allow many others to mount them frequently during estrus (Refsal and Seguin, 1980; Galina et al., 1982). The number of cows in standing estrus at the same time effected the frequency of mounting and duration of estrus in dairy cattle ( Hurnik et al., 1975; Helmer and Britt, 1985; Pennington et al., 1985; Walton et al., 1987; Van Vliet and Van Eerdenburg, 1996). Dairy cows in a free stall area exhibited an average of 11.2 mounts per estrus during 7.5 h with one estrus cow, which increased to 52.6 mounts during 10.1 h when three cows were in estrus at the same time (Hurnik et al., 1975). Similarly, in a free stall facility with slatted flooring, the number of mounts per dairy cow increased ( $P < 0.004$ ) from an average of 3.5 mounts in 2.5 h with one estrous cow, to 40 mounts in 17 h with four cows in estrus at the same time (Walton et al., 1987). Mounts per cow increased by 9.9 and duration of standing estrus increased by 3.7 h for every additional dairy cow in estrus, simultaneously, from one to six cows (Pennington et al., 1985). In a group of nine dairy heifers, the number of mounts increased ( $P < 0.05$ ) as the number of cows in estrus at the same time increased from one ( $4.5 \pm 1.2$  mounts per estrus) to four ( $39.0 \pm 5.5$  mounts per estrus; Helmer and Britt, 1985). Bull interaction can alter estrous behavior. Bulls first become interested in cows about 4 d before estrus (French et al., 1989) and take initiative in guarding and then mounting the cow. However, if bulls are newly introduced to a herd of cows, they may spend more time butting other cows to establish dominance than detecting estrual cows (Orihuela et al., 1983). At times a heifer may stand only for other females to mount and not stand for the bull (Plasse et al., 1970).

Bulls performed more mounts during the day, while homosexual activity increases at night (Orihuela et al., 1983).

### Methods to Detect Estrus

Many approaches have been developed to increase the ability to detect cows in estrus. While continuous observation is very accurate, it is not practical for estrus detection by typical producers. Several observations throughout the day for 30 min to an hour may detect most cows in estrus, but efficiency of estrous detection increased by 10 to 20% when observation periods were added at 1200 h and 2400, respectively, to observations at 0600 and 1800h (Hall et al., 1959). Detection of estrus for one hour at 0700, 1200, and 1600 h detected 91% of dairy heifers in estrus, and without the 1200 h observation, 90% were detected (Donaldson, 1968). The number of cows in estrus detected with visual observation can be improved with the addition of tail chalking or painting, mount detector patches, pedometers, modified bulls or androgenized cows or steers. Tail chalking or painting the tailhead region with a grease chalk or paint, allows producers to identify cows that have been mounted without actually observing the mount. When mounted by another animal, the chalked area begins to rub off. With skilled interpretation of chalk removal, detection results are as good as with mount detector patches (Williamson, 1980). When an aerosol raddle paint strip of contrasting color was applied over the tail paint, 94.5% of dairy heifers were detected in estrus (Macmillan et al., 1988). A plastic patch with a dye filled reservoir, know as a mount detector patch, can be attached to the rump area. When the patch receives adequate pressure from mounting animals the dye will be forced from the reservoir and color the rest the patch. If an animal is only mounted a few times, tail chalking and mount detector patches may

not be adequately activated to detect the animal in estrus. More ( $P < 0.05$ ) estrous periods were detected for dairy cows with the use of chalk plus a mount detector patch (63.8%) or with a mount detector patch (56.6%), than with chalk (44.0%) or three 30 min visual observation periods (42.5%; Pennington and Callahan, 1986). Tail chalking and mount detector patches can be used in conjunction with a modified bull or an androgenized animal. Modified bulls are either vasectomized (Plasse et al., 1970) so that sperm are not ejaculated or bulls are surgically modified so that intromission cannot occur. In both cases the bull maintains an active libido and will seek out and mount cows in estrus. Estrus was detected 93% of the time in dairy heifers that were teased with a vasectomised bull for an hour, during three times a day, which was similar to 1 h visual observation 3 times a day (90.4%; Donaldson, 1968). Androgenized animals are also used to identify estrous cows (Gwazdauskas et al., 1990). Cows and steers can be treated with testosterone to increase mounting activity (Mortimer et al., 1990; Nix et al., 1998). Both modified bulls and androgenized animals can be fitted with a chinball marker to allow marking of estrous cows. A chinball marker is a halter type device that fits on the animals head. Located on the halter underneath the chin, is a ball point pen type of marker, that will leave a line of ink or paint when contacting an object. Marks on the back or shoulder area cows can be identified as marks associated with mounting. Marks associated with mounting cannot always be distinguished from marks caused by chin resting on an estrus or nonestrus cows. Surgically altered bulls with chinball markers identified 87% of dairy heifers in estrus (Foote, 1975) and androgenized cows detected 74% of beef cows in estrus (Kiser et al., 1977). Accuracy of estrous detection rates were similar in dairy cows with mount detector patches (66%), cows marked by an androgenized female with a chin ball marker (79%), and those observed in standing

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estrus (68%; Stevenson and Britt, 1977). Accuracy of estrous detection was increased when standing was included with the other two methods (standing and paint patch, 100%; standing and marked, 88%; Stevenson and Britt, 1977).

Pedometers are devices attached to the leg of a cow to measure activity by recording the number of steps taken. Because they need to be read at least once a day, they are practical in dairy operations but not for beef cattle. As detected with pedometers, ambulation increases from 100% to 393% on the day of estrus (Kiddy, 1976; Lewis and Newman, 1984; Pennington et al., 1986; Redden et al., 1993). Pedometers consistently predicted more ovulations with greater accuracy as days postpartum increased than twice daily observation for 30 min in dairy cows (Peter and Bosu, 1986). Estrus in heat stressed and sick animals may be difficult to detected with pedometers.

Two radiotelemetric computerized systems have been developed to detect estrus without daily handling of cows. Some labor is involved in securing and maintaining the devices to the animal. The first is a system that monitors the changes in vaginal temperature associated with estrus through a small device inserted into the vagina that takes temperature readings and sends them to a computer which records them every four minutes. Monitoring of vaginal temperature had higher prediction sensitivity ( $88.4 \pm 9.6$ ) but more false positives than visual observation for 20 min/h for 4 h in the morning with casually observations 4 to 6 times throughout the day (Kyle et al., 1998). Similarly, Redden et al., (1993) found that vaginal temperature was comparable to pedometry measurements, and both were superior to daily observation of estrus in an exercise area. The HeatWatch<sup>®</sup> system is a pressure sensitive rump mounted device that records the date, time and duration of mounts received using radio telemetry through a computer. HeatWatch<sup>®</sup> and vaginal temperature monitors require computer data to be analyzed on at

least a daily basis. HeatWatch® system has an efficiency of 89.5 to 100% and accuracy of 78 to 100% (Stevenson et al., 1996b; Timms et al., 1997; Xu et al., 1998).

HeatWatch® detected more ( $P < 0.01$ ) cows in estrus than visual observation (87.8 vs 63.5%; Greene and Borger, 1996) while the detection rate was similar ( $P > 0.10$ ) to using visual observation with tailpainting (Xu et al., 1998). Both mount detector patches and HeatWatch® patches must be carefully attached so that they are not rubbed off or lost due to shedding of hair.

A better understanding of factors that influence estrous behavior in beef cows could increase the efficiency of estrous detection and reduce the time and labor needed for AI. This would encourage more producers to utilize this technology and increase the rate of genetic progress. Although it is established that increasing the number of dairy cows in estrus at the same time increases the duration and number of mounts per estrus, this behavior response has not been explored in beef cows. It has been suggested that confinement area may influence estrous behavior of beef cows but the effect of pen size has not been established. The use of estrous detection aids may increase the efficiency of identification of cows in estrus.

CHAPTER III  
EFFECTS OF NUMBER OF COWS IN ESTRUS  
AND CONFINEMENT AREA ON ESTROUS BEHAVIOR  
IN BEEF COWS

ABSTRACT: Non-lactating Angus and Angus x Hereford cows were studied in winter (December and January) and summer (July and August), to determine the effects of confinement area and number of cows in estrus on estrous behavior. During each season, 16 cows were maintained either in a drylot (60 x 100 m) or a pasture (12 ha). Estrous cycles were synchronized with two injections of prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) 10 to 14 d apart at the initiation of the experiment. Thereafter,  $PGF_{2\alpha}$  was administered on d 6 to 18 of the estrous cycle so that 1, 2 or 3, 4 to 6, or 7 or more cows were estrus at the same time. Blood samples were collected at treatment with  $PGF_{2\alpha}$ , and at 4 and 10 d after  $PGF_{2\alpha}$  and concentration of progesterone in plasma were quantified by RIA. If progesterone in plasma was  $> 1$  ng/mL at  $PGF_{2\alpha}$  treatment,  $< 1$  ng/mL 4 d post  $PGF_{2\alpha}$  treatment, and  $> 1$  ng/mL 10 d post  $PGF_{2\alpha}$  treatment, cows were classified as having a normal luteal response to  $PGF_{2\alpha}$  and were used in analyses. Duration of estrus and the number of mounts during estrus were recorded by HeatWatch<sup>®</sup>. If any part of estrus for a cow occurred at the same time as another cow, the cow was considered to influence the estrous behavior of the other cow. Cows in the drylot had a shorter ( $P < 0.02$ ;  $61.8 \pm$

3.1 h) interval to estrus after PGF<sub>2α</sub> treatment than cows on pasture (72.8 ± 3.3 h). The interval to estrus was longer (P < 0.07) when cows were treated with PGF<sub>2α</sub> on d 10 to 13 (76.7 ± 3.7) of the estrous cycle than when treated on d 6 to 9 (62.3 ± 4.7 h) or d 14 to 18 (62.9 ± 3.6 h). Increasing the number of cows in estrus at the same time, increased the number of mounts per estrus (P < 0.0001) and the duration of estrus (P < 0.01). When one cow was estrus, she was mounted 11.0 ± 6.2 times during 11.6 ± 1.5 h. When seven or more cows were in estrus at the same time, each cow received an average of 50.4 ± 3.2 mounts per estrus during 17.3 ± 0.8 h. Cows were in estrus longer (P < 0.01) in winter (16.8 ± 0.7 h) with more (P < 0.06) mounts (34.2 ± 2.8) than in summer (13.9 ± 0.8 h; 25.7 ± 3.4 mounts). Cows in drylot were estrus longer (P < 0.05; 16.7 ± 0.8 h) than cows on pasture (14.2 ± 0.7 h). Duration of the longest interval between mounts decreased (P < 0.002) as the number of cows in estrus at one time increased (5.3 ± 0.7 h for one estrus cow, 2.6 ± 0.3 h when seven or more cows were estrus). We conclude that increasing the number of cows in estrus at the same time will increase the number of times a cow is mounted and the duration of estrus. The increase in estrous behavior associated with more cows in estrus could increase the number of estrous cows detected with infrequent visual observation.

KEYWORDS: Beef cow, estrus, HeatWatch<sup>®</sup>, pen size, PGF<sub>2α</sub>

## Introduction

Artificial Insemination (AI) allows the introduction of superior genetics that may not be available with natural service. However, AI has not been widely implemented throughout the beef industry. In 1997, only 7.1% of beef operations used AI (USDA,

1998). The additional time and labor required and complications that it introduces are the two most common reasons that producers do not use AI (USDA, 1997). Detection of estrus is a major contributor to additional time required to implement AI.

Estrous behavior varies with the type of animal and management system. Synchronized beef heifers were in estrus for 8 to 15 h and received 27 to 50 mounts per estrus when monitored continuously with the HeatWatch<sup>®</sup> system (Stevenson et al., 1996b; Lemaster et al., 1999; Rae et al., 1999). However, beef cows received fewer mounts and were in estrus for a shorter time at the first postpartum estrus (Ciccioli and Wettemann, 2000; Lents, 2001). Adequate detection of estrus can be accomplished by visual observations. Several observations throughout the day detected 77 to 90% (Donaldson, 1968; Stevenson et al., 1996b) of cows in estrus and efficiency of estrous detection increases when the number of observation periods is increased (Hall et al., 1959). Twenty-seven percent of estruses in beef heifers were not detected with twice daily visual observation (Stevenson, 1996b).

Investigation of factors that can reduce required labor and increase the percentage of cows detected in estrus is needed. Increasing the number of dairy cows in standing estrus at the same time increased the frequency of mounting and duration of estrus (Humik et al., 1975; Helmer and Britt, 1985; Pennington et al., 1985; Walton et al., 1987; Van Vliet and Van Eerdenburg, 1996;) and confinement area may influence estrous behavior (Mattoni, 1988). A better understanding of factors that influence estrous behavior in beef cows could facilitate the use of AI. Our objective was to determine the effects of the number of cows in estrus and confinement area on estrous behavior of beef cows.

## Materials and Methods

### Animals and Animal Management

Nonlactating Angus and Angus x Hereford ( $n = 32$ ) cows that were  $4.1 \pm 1.4$  years (range 2.5 - 10), weighed  $541.4 \pm 5.1$  kg and had body condition score of  $5.8 \pm 0.8$  (1 = emaciated, 9 = obese; Wagner et al., 1988), were used in two replications during winter (January and February, 2000) and summer (July and August, 2000) in Oklahoma. At the beginning of treatment, cows were paired by BCS and age and were allotted to either a 60 x 100 m drylot or a 12 ha pasture. There were sixteen cows in each confinement area during each season. Six cows from the winter did not respond to synchronization in the summer and were replaced with cows of similar age, weight and BCS. The pasture group grazed native Oklahoma grasses. Both groups were given a 38% crude protein supplement and prairie hay as needed to maintain body condition. The drylot had a 2.7 x 5.5 m artificial shade in the winter, which was extended in the summer to 2.7 x 10 m, and the pasture group had adequate trees for shade. The drylot had an automatic water fountain and the pasture had a metal tank for water without access to ponds for cooling.

### Treatment

Cows were synchronized with two injections of  $\text{PGF}_{2\alpha}$  (Lutalyse<sup>®</sup>, 25 mg Pharmacia & UpJohn, Kalamazoo, MI) at a 10 to 14 d interval at the beginning of treatment. After initial synchronization, cows were given one injection of  $\text{PGF}_{2\alpha}$  between d 7 and d 18 (d 0 = estrus) of the estrous cycle to create groups of 1, 2, 4, or 6 cows in estrus on a day (Table 3). Blood samples were collected immediately prior to

Table 3. Schedule of treatment with PGF<sub>2α</sub> to induce estrus in different numbers of cows at the same time

Cow no.	Day of Treatment																	
	-10	0	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40
1	X	X			X					X								
2	X	X			X								X					
3	X	X			X										X			
4	X	X			X						X							
5	X	X	X						X						X			
6	X	X		X					X						X			
7	X	X				X					X							
8	X	X				X									X			
9	X	X				X									X			
10	X	X				X						X						
11	X	X						X						X				
12	X	X						X										X
13	X	X					X					X						
14	X	X					X									X		
15	X	X					X								X			
16	X	X					X										X	

X Treatment with PGF<sub>2α</sub>

treatment with  $\text{PGF}_{2\alpha}$ , and at 4 and 10 d after treatment. Each cow was treated with  $\text{PGF}_{2\alpha}$  2 or 3 times after initial synchronization, and each treatment was given so that the cow would be in estrus with a different number of cows than the previous time(s) she was treated. Cows in the pasture were gathered no more than one hour before the cows to be treated were removed from the group, and non-treated cows were returned to pasture. Cows were removed from the group for less than 1.5 h during treatment and bleeding and returned to the group immediately after all treatments were complete. There were always at least four cows in a subgroup so that estrus could be detected while treated cows were removed from the group. Treatment in the summer was given at approximately 0900 and treatment in the winter was given at approximately 1100.

#### Progesterone

Blood was collected in tubes containing EDTA by tail venipuncture and cooled to 4° C. Samples were centrifuged for 15 min at 2500 x g within 2 h of collection. Plasma was aspirated and stored at -20° C until analysis. Solid phase RIA (Coat-A-Count progesterone kit, Diagnostic Products Corp., Los Angeles, CA; Vizcarra et al., 1997), was used to quantify concentrations of progesterone in plasma. Only estrous cows that had greater than 1 ng/mL of progesterone in plasma at the time of  $\text{PGF}_{2\alpha}$  administration, less than 1 ng/ml in plasma at 4 d after  $\text{PGF}_{2\alpha}$ , and greater than 1 ng/mL in plasma at 10 d after  $\text{PGF}_{2\alpha}$  were considered normal and were used to quantify estrous behavior (Garverick et al., 1971; Swanson et al., 1972; Wettemann et al., 1972). When cows exhibited estrous behavior but did not have normal concentrations of progesterone in plasma the cows were considered to influence the behavior of others but their estrous



behavior was not quantified.

## Estrus

The HeatWatch<sup>®</sup> (DDx Inc., Denver, CO) system was used to monitor estrous behavior. A pressure sensor and radio transmitter were enclosed in a nylon patch and attached to the rump of each cow with industrial strength glue (OSI Quickbond<sup>®</sup>; Ohio sealants Inc., Mentor, Ohio) just prior to the second treatment with PGF<sub>2α</sub> of the initial synchronization. During treatment, cows were observed at least once each day to determine that patches were attached to the rump of the cows. Patches were evaluated at each treatment and sample collecting to determine that they were firmly attached to the rump of the cow, and reattached if necessary, to prevent loss of patches due to shedding of hair. A computer recorded the date, time and duration of all mounts greater than or equal to two seconds. Manual activation of extra pressure sensors was conducted three times a week to ensure proper function of the HeatWatch<sup>®</sup> system.

Visual observation of estrus was conducted to determine the ability of HeatWatch<sup>®</sup> to accurately record mounting activity when many cows were in estrus. The drylot cows were continuously observed for 28 h during initial synchronization in summer. Cows were marked with grease markers of differing colors and cow color, markings and ear tag number were recorded to aid in identification of animals. Cows were observed from a 1.7 m high platform adjacent to the drylot, and two observers recorded the time of all mounts. Individuals observed cows for 4 h shifts, and shift changes occurred every two hours, so that there was always one observer that had been with the cows the previous two hours and was familiar with the animals in estrus. Number of mounts determined by HeatWatch<sup>®</sup> was correlated with mounts detected

visually ( $r = .91$ ;  $P < 0.0001$ ; Figure 1). Every estrus detected visually was also detected by HeatWatch<sup>®</sup>. However, the number of mounts detected by HeatWatch<sup>®</sup> was less ( $P < 0.008$ ) than those detected visually (18.4 % less). When fewer than 40 mounts per estrus were recorded visually, HeatWatch<sup>®</sup> recorded 8.9 % fewer mounts than visual observation. When more than 40 mounts were recorded, HeatWatch<sup>®</sup> recorded 22.5 % fewer mounts than visual observation.

The onset of estrus was the first of two mounts that occurred less than 4 h apart. The end of estrus was the last mount with no mounts in the following 12 h, but to insure that the last mount was actual estrous behavior it had to be preceded by a mount in the previous 4 h. Duration of estrus, number of mounts, the longest interval between subsequent mounts and whether the longest interval between mounts occurred in the first or last half of estrus were determined. When any part of estrus for cows overlapped, the cows were considered to affect the estrous behavior of each other. The day of the cycle that each PGF<sub>2α</sub> treatment was given was determined from the last exhibited estrus ( $d = 0$ ) and verified by the concentration of progesterone in plasma. The interval from PGF<sub>2α</sub> treatment until estrus was determined in hours.

### Statistical Analyses

The effect of stage of the cycle ( $d 6$  to  $9$ ,  $d 10$  to  $13$ ,  $d 14$  to  $18$ ) at PGF<sub>2α</sub> administration on response to treatment, the interval to estrus and the mounts per estrus was determined with a completely randomized design using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model, included stage of the cycle at treatment, season (summer or winter), confinement area (drylot or pasture), and number of cows in estrus (1-16) was a covariate. Means were compared by Scheffe's procedure (Steel and

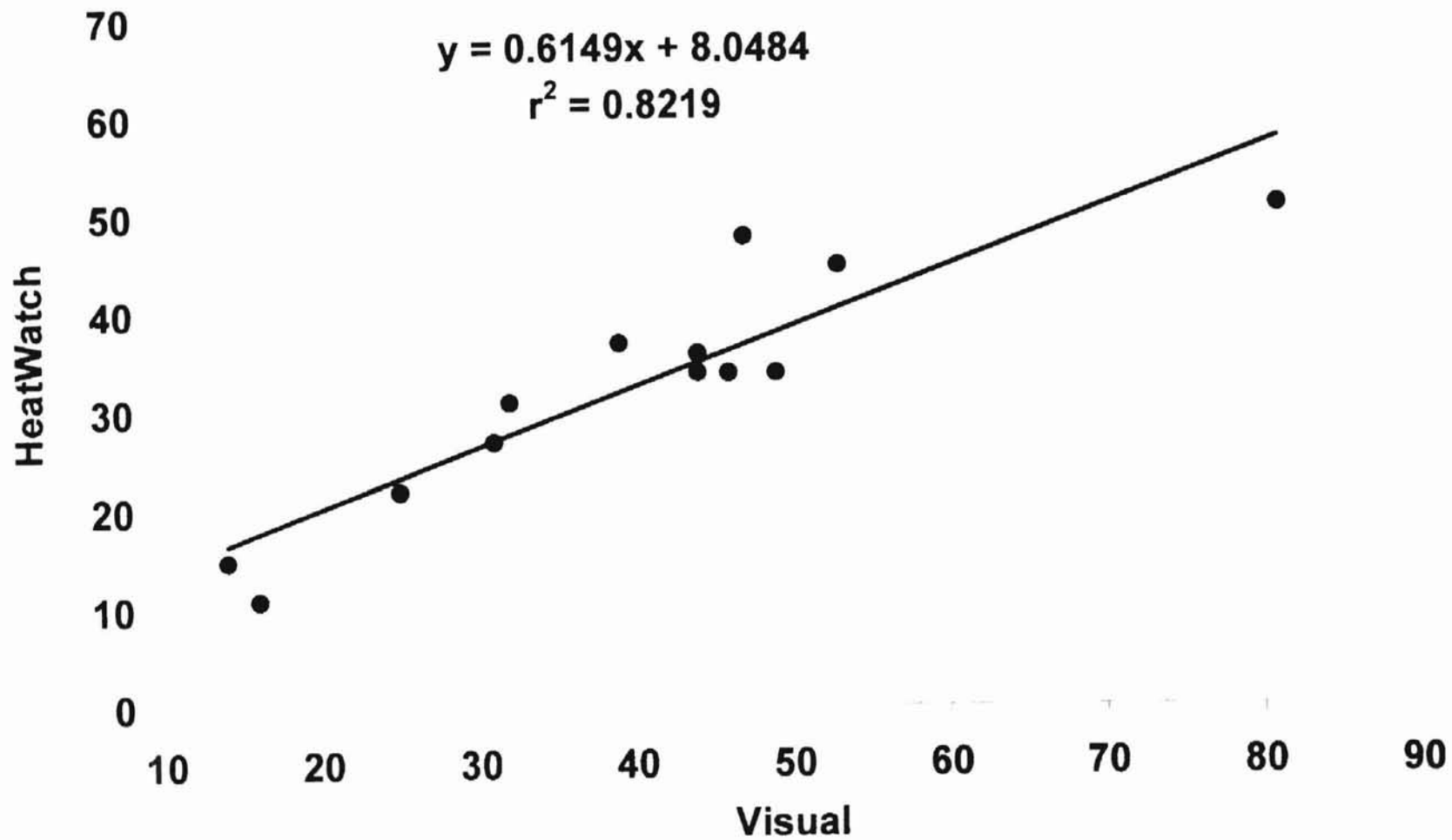


Figure 1. Mounts detected with HeatWatch and visual observation ( $P < 0.001$ ).

Torrie, 1980). Correlations between the interval to estrus after  $\text{PGF}_{2\alpha}$  with the number of mounts and duration of estrus were determined with the PROC CORR procedure of SAS.

The effects of season, confinement area, and the number of cows in estrus (1, 2 to 3, 4 to 6, 7 or more) on the number of mounts, duration of estrus, longest interval between mounts and whether the longest interval between mounts was in the first or last half of estrus were determined with a completely randomized design using the PROC GLM procedure of SAS. The model included season, confinement area, the number of cows in estrus, and all two-way interactions. Scheffe's test was used to compare means when treatment was significant ( $P < 0.05$ ). Correlations between duration of estrus with the number of mounts per estrus and number of cows in estrus and between number of mounts and number of cows in estrus and the duration of estrus were determined with the PROC CORR procedure of SAS.

## Results

### Response to $\text{PGF}_{2\alpha}$

Ninety-four (59 of 63) percent of cows with a functional CL had estrous behavior with normal concentrations of progesterone in plasma after synchronization with  $\text{PGF}_{2\alpha}$  at the initiation of the experiment. Luteal regression did not occur in two cows in the winter and two cows in the summer did not have new luteal development after the initial estrous synchronization (Table 4). Estrous behavior associated with abnormal luteal responses after the initial synchronization, or after subsequent treatment with  $\text{PGF}_{2\alpha}$ , was excluded from estrous analyses, but these cows were considered to influence the behavior

of other cows in estrus. Stage of the cycle at treatment was not determined at the initial synchronization of cows.

After the initial synchronization, 81.3% of 134 cows with a functional CL at treatment with PGF<sub>2α</sub> (d 0) exhibited estrus in 2 to 5 d after PGF<sub>2α</sub> and had < 1 ng/mL of progesterone on d 4, and > 1 ng/mL on d 10. Luteal regression and estrus did not occur after treatment in two cows in winter and three cows in summer (Table 4). Based on concentrations of progesterone in plasma, six cows (4.5%) ovulated after PGF<sub>2α</sub> treatment without behavioral estrus. During the winter on pasture, one cow with normal luteal regression after treatment with PGF<sub>2α</sub> was in estrus for over 64 h and was excluded from all analyses as an outlier (mean ± 3 SD). One cow in the summer drylot group was excluded from analyses because she had a split estrus (2 mounts within 14 min., then over 18 h with no mounts, followed by 3 mounts in about 2 h.). Two cows during the winter and ten cows during the summer had normal luteal function at treatment (P4 > 1 ng/mL) and luteal regression after treatment (P4 < 1 ng/mL at 4 d post treatment) but did not develop a normal CL after luteolysis (P4 remained < 1 ng/mL for 10 to 20 d after PGF<sub>2α</sub> treatment). Estrus occurred in nine of the twelve cows that had luteal regression after PGF<sub>2α</sub>, but did not form a functional CL.

Ninety-six (127 of 132) percent of cows with a functional CL at PGF<sub>2α</sub> treatment after the initial synchronization had luteal regression. In nine percent (12 of 132) of the cases luteal regression was not followed by new luteal development. Of cows that had luteal regression, 92% exhibited estrous behavior. Season and day of the estrous cycle at treatment (d 6 to 9, 10 to 13, or 14 to 18) did not influence ( $P > 0.10$ ) the percentage of cows with a functional CL that had luteal regression after PGF<sub>2α</sub> treatment, or the

Table 4. Estrus and luteal response after treatment of beef cows with PGF<sub>2α</sub>

Response to PGF <sub>2α</sub> Treatment				
	Normal <sup>c</sup>	Ovulation without estrus <sup>d</sup>	Lacked luteal regression and estrus <sup>e</sup>	Lacked new luteal development <sup>f</sup>
<b>Winter</b>				
Pasture				
Initial synchronization <sup>u*</sup>	15	0	0	0
PGF <sub>2α</sub> <sup>b+</sup>	30	3	0	0
Drylot				
Initial Synchronization	14	0	2	0
PGF <sub>2α</sub> <sup>*</sup>	28	1	2	2
<b>Summer</b>				
Pasture				
Initial Synchronization	15	0	0	1
PGF <sub>2α</sub> <sup>*</sup>	23	2	2	6
Drylot				
Initial synchronization	15	0	0	1
PGF <sub>2α</sub> <sup>-</sup>	28	0	1	4
<b>Totals</b>				
Initial Synchronization	59	0	2	2
PGF <sub>2α</sub>	109	6	5	12

\* one cow omitted from total for absence of CL at treatment.

+ one cow omitted from total as an outlier ( $\pm 3$  standard deviations).

- one cow omitted from total for split estrus (over 18 h separating mounting behavior)

<sup>u</sup> Estrus within 2 to 5 d after the second of two treatments with PGF<sub>2α</sub> at a 10 to 14 d interval

<sup>b</sup> Response to single PGF<sub>2α</sub> treatment when cows had luteal function (progesterone > 1 ng/ml at treatment).

<sup>c</sup> Progesterone > 1 ng/mL at treatment, < 1 ng/mL 4 d after treatment, and > 1 ng/mL 10 d after treatment and received  $\geq 2$  mounts 2 to 5 d after PGF<sub>2α</sub> treatment.

<sup>d</sup> Progesterone > 1 ng/mL at treatment, < 1 ng/mL 4 d after treatment, > 1 ng/mL 10 d after treatment and received < 2 mounts 2 to 5 d after PGF<sub>2α</sub> treatment.

<sup>e</sup> Progesterone > 1 ng/mL at treatment and > 1 ng/mL 4 d after treatment and < 2 mounts.

<sup>f</sup> Progesterone > 1 ng/mL at treatment, < 1 ng/mL 4 d after treatment, and < 1 ng/mL 10 d after treatment with or without mounts at 2 to 5 d after PGF<sub>2α</sub> treatment.

percentage of cows that exhibited estrus.

Confinement area and day of the estrous cycle at treatment (d 6 to 9, 10 to 13, or 14 to 18) influenced the interval to estrus but the interaction was not significant. Cows in drylot had a shorter interval to estrus ( $P < 0.02$ ;  $61.8 \pm 3.1$  h) after  $\text{PGF}_{2\alpha}$  treatment than cows on pasture ( $72.8 \pm 3.3$  h). The interval to estrus was longer ( $P < 0.07$ ; Figure 2) when cows were treated on d 10 to 13 of the estrous cycle ( $76.7 \pm 3.65$  h) than when treated on d 6 to 9 ( $62.3 \pm 4.67$  h) or d 14 to 18 ( $62.9 \pm 3.58$  h).

Day of the estrous cycle at  $\text{PGF}_{2\alpha}$  treatment did not influence ( $P > 0.5$ ) the percentage of cows responding to treatment (d 6 to 9, 77.4%; d 10 to 13, 86.7%; d 14 to 18, 83.3%). Cows treated with  $\text{PGF}_{2\alpha}$  on d 6 to 9 and d 14 to 18 had a greater synchrony of estrus than those treated on d 10 to 13 (Figure 3). More ( $P < 0.007$ ) cows treated on d 6 to 9 (75%) and on d 14 to 18 (53%) of the cycle initiated estrus between 40 and 60 h after treatment than cows treated on d 10 to 13 of the cycle (34%). The greatest percentage of cows treated on d 10 to 13 that initiated estrus during a 20 h period was 40% during 50 to 70 h after treatment. A similar percentage of cows treated on d 6 to 9 of the cycle (35%) responded during 50 to 70 h after treatment as those treated on d 10 to 13, but more ( $P < 0.01$ ) cows treated during d 14 to 18 (73%) of the cycle responded during that period. Day of the cycle at  $\text{PGF}_{2\alpha}$  treatment did not influence ( $P > 0.10$ ) the duration of estrus or the number of mounts per estrus. However, the interval to estrus after  $\text{PGF}_{2\alpha}$  treatment was correlated with the duration of estrus ( $r = -0.16$ ,  $P < 0.05$ ) and the number of mounts per estrus ( $r = -0.20$ ,  $P < 0.02$ ).

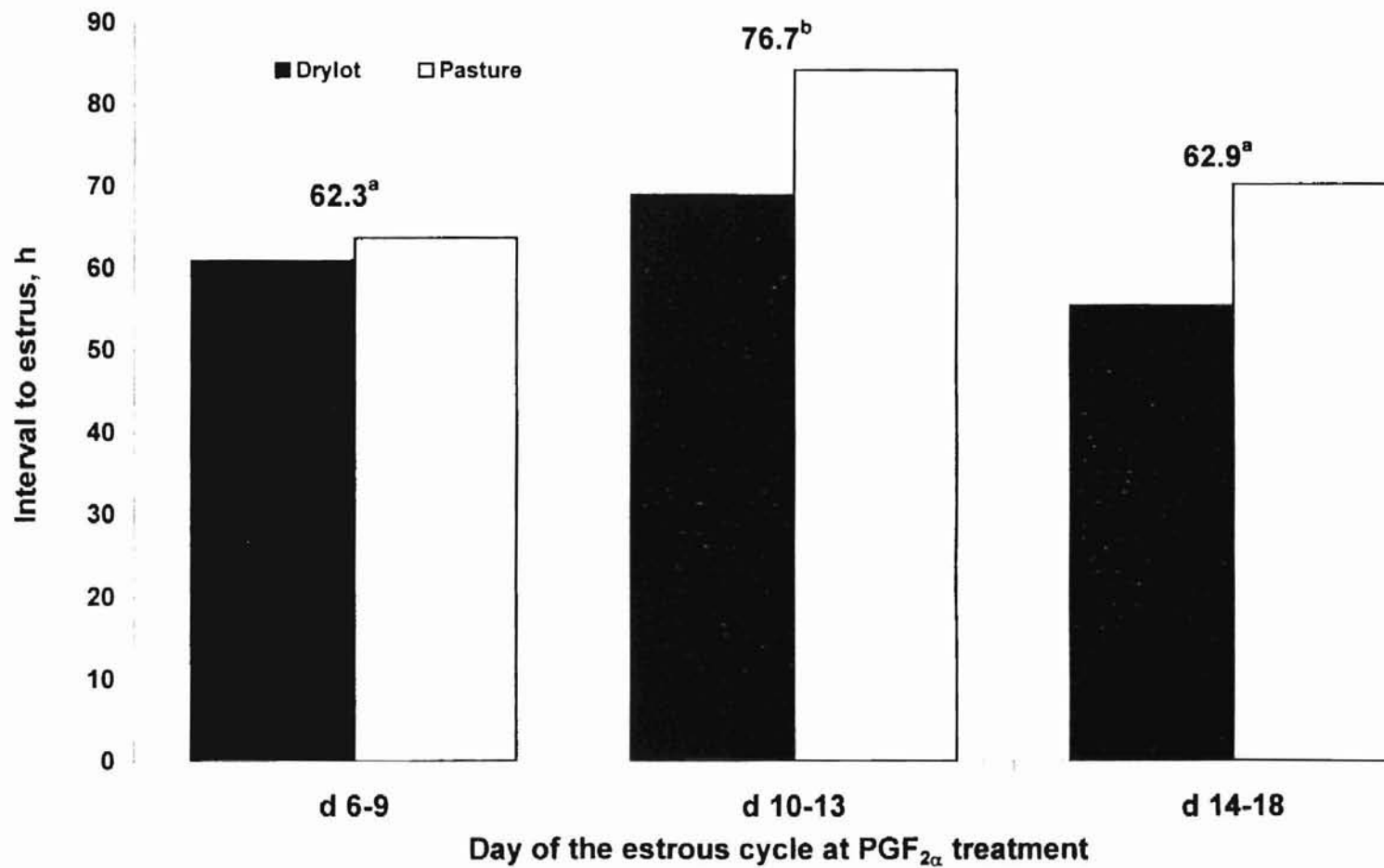


Figure 2. Influence of confinement area and day of the estrous cycle at treatment on the interval from PGF<sub>2α</sub> treatment to estrus (confinement area,  $P < 0.02$ ; day of the cycle  $P < 0.02$ ). <sup>a,b</sup> differ  $P < 0.07$ .



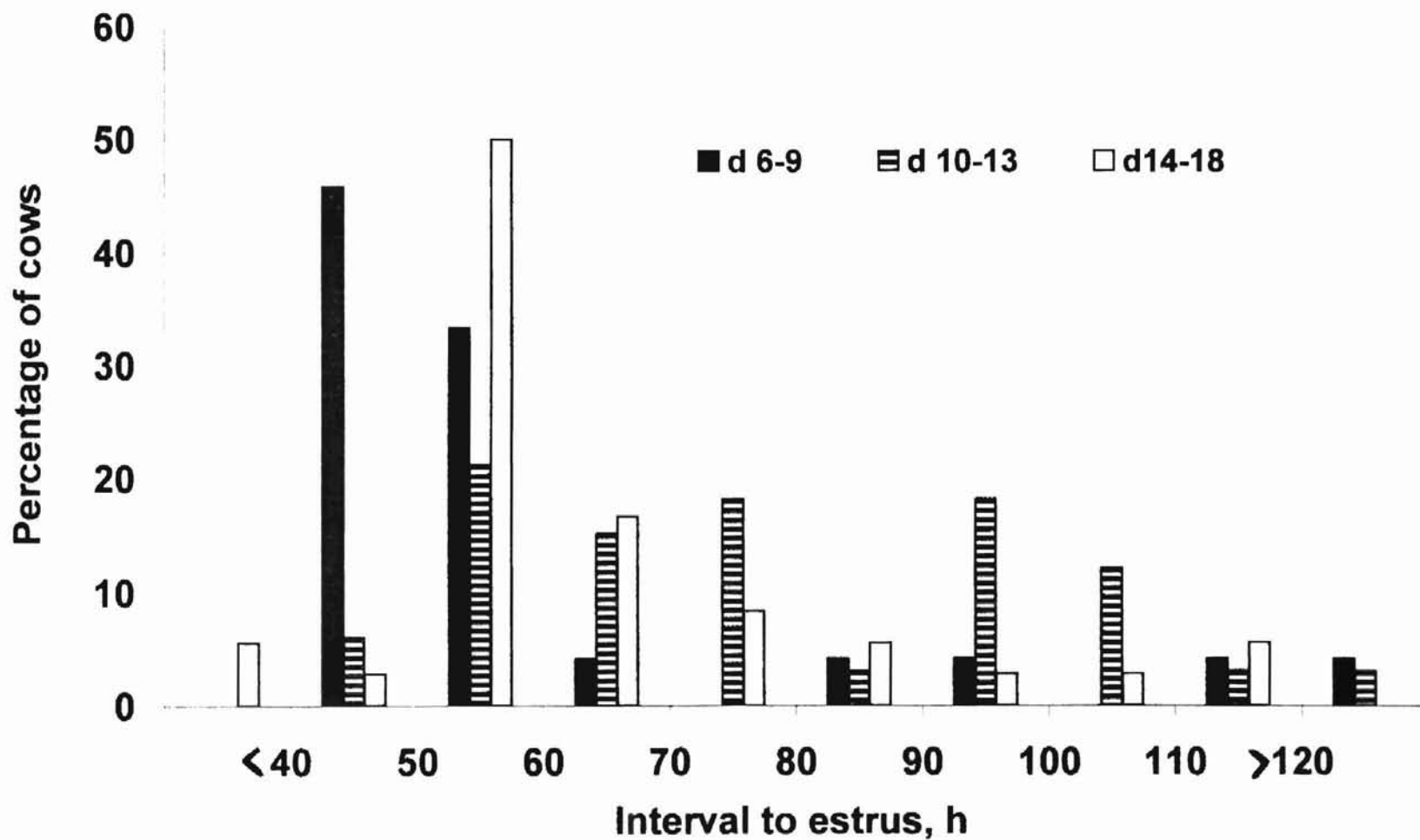


Figure 3. Influence of day of estrous cycle at PGF<sub>2α</sub> treatment on the percentage of cows with onset of estrus during various intervals.

## Estrous Behavior

The number of observations for each behavioral group with different numbers of cows in estrus, and the number of times each group occurred for observation during the experiment, are summarized in table 5. The number of cows in estrus ( $P < 0.0001$ ) and season ( $P < 0.06$ ) influenced the number of mounts per estrus, but number of mounts per estrus was not influenced ( $P > 0.10$ ) by confinement area or interactions among confinement area, number of estrous cows and season. Number of mounts per estrus cow increased as the number of cows in estrus at the same time increased. When only one cow was estrus, she received  $11.0 \pm 6.2$  mounts, which increased ( $P < 0.07$ ) to  $30.9 \pm 3.6$  mounts per estrus when 4 to 6 cows were in estrus (Figure 4). When two or three cows were in estrus together, the number of mounts each cow received ( $27.7 \pm 3.6$ ) was intermediate and not different from when one cow or 4 to 6 cows were in estrus. Seven or more cows in estrus received more ( $P < 0.002$ ) mounts per estrus ( $50.4 \pm 3.2$ ) than when 4 to 6 cows were in estrus. When only one cow was in estrus, 47% of the cows had between 2 and 10 mounts (Figure 5). More than 60% of cows were mounted greater than 20 times when two or more cows were in estrus. When seven or more cows were in estrus, more than 77% of the cows were mounted greater than 30 times per estrus. Cows received more ( $P < 0.06$ ) mounts per estrus in winter ( $34.2 \pm 2.8$  mounts) than in summer ( $25.7 \pm 3.4$  mounts; Figure 6). During both seasons, the number of mounts per estrus ranged from two when 1 to 4 cows were in estrus to 166 when all sixteen cows were in estrus.

Length of estrus was affected by season ( $P < 0.01$ ), number of cows in estrus ( $P < 0.01$ ) and confinement area ( $P < 0.05$ ), with no interactions between main effects. When the number of cows in estrus increased from one to four to six, the duration of estrus

Table 5. Number of observations for each behavioral group with different numbers of cows in estrus, and the number of times each size group occurred<sup>a</sup> during season and confinement

	Number of cows in estrus			
	1	2-3	4-6	>7
<b>Winter</b>				
Pasture				
Number of observations	8*	17	9	11
Number of times the group occurred	8	10	5	5
Drylot				
Number of observations	5	6*	15	13
Number of times the group occurred	5	5	8	7
<b>Summer</b>				
Pasture				
Number of observations	2	9	11	14
Number of times the group occurred	2	7	6	9
Drylot				
Number of observations	3	15	8*	15
Number of times the group occurred	3	8	6	9
<b>Totals</b>				
Number of observations	18	47	43	53
Number of times the group occurred	18	30	25	30

<sup>a</sup> Groups were considered different when more than half of the cows in estrus changed.

\* One less observation for mounts than duration due to missing data.

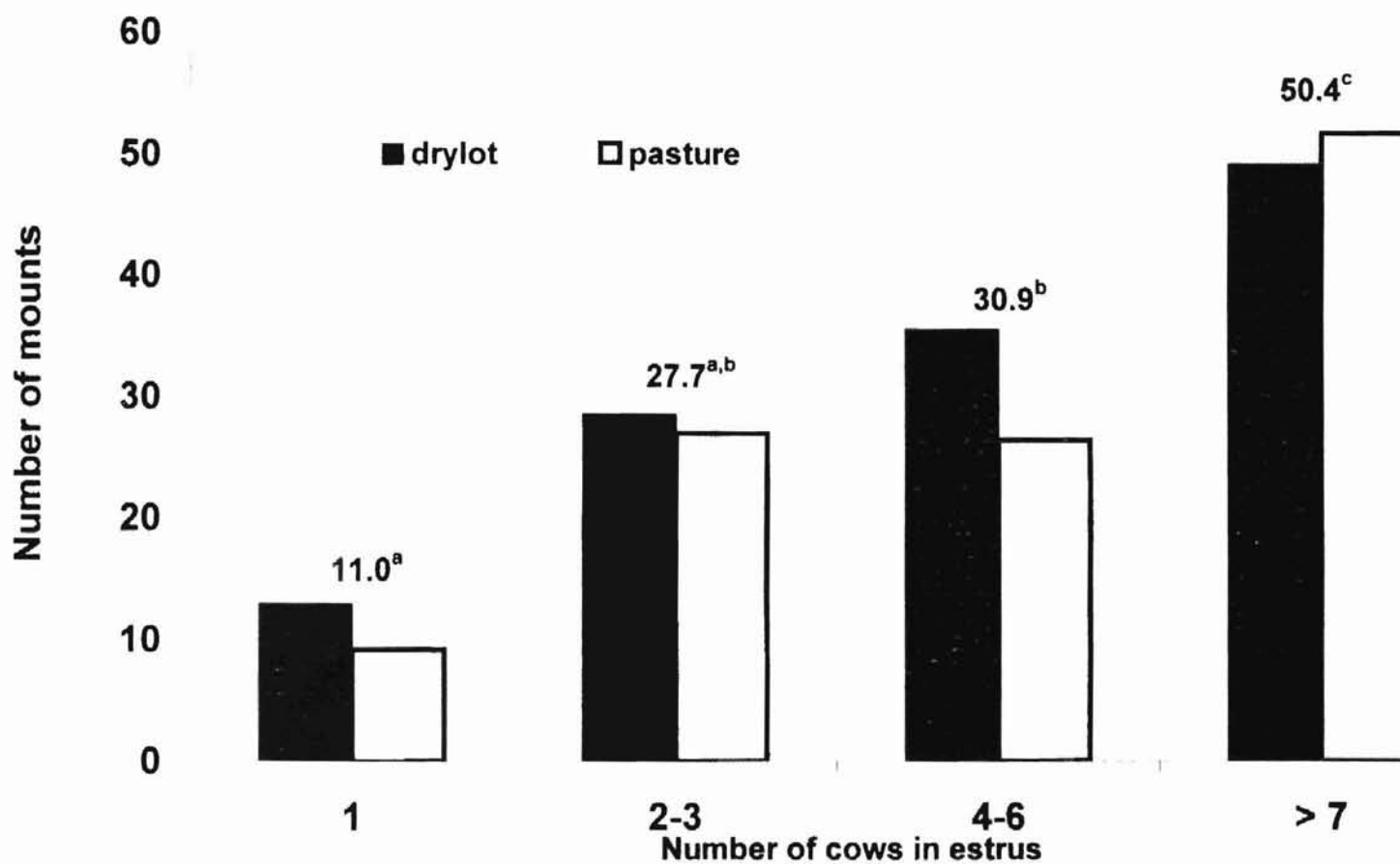


Figure 4. Influence of number of cows in estrus on number of times cows were mounted in drylot and pasture. (Cows in estrus,  $P < 0.001$ ; Confinement,  $P > 0.4$ ).

<sup>a,b</sup> differ  $P < 0.07$ ; <sup>b,c</sup> differ  $P < 0.002$ .

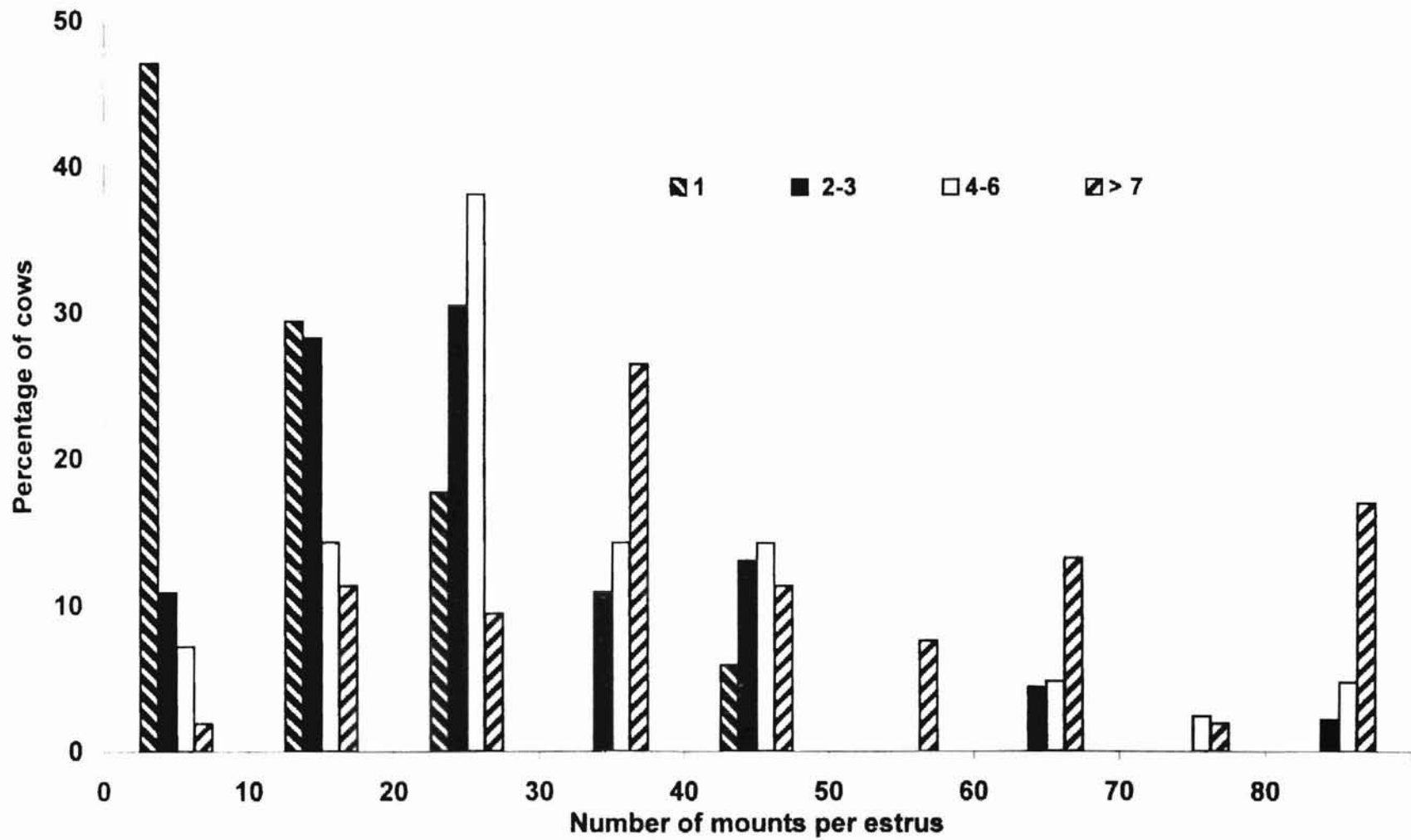


Figure 5. Influence of number of cows in estrus on the percentage of cows with different numbers of mounts during estrus.

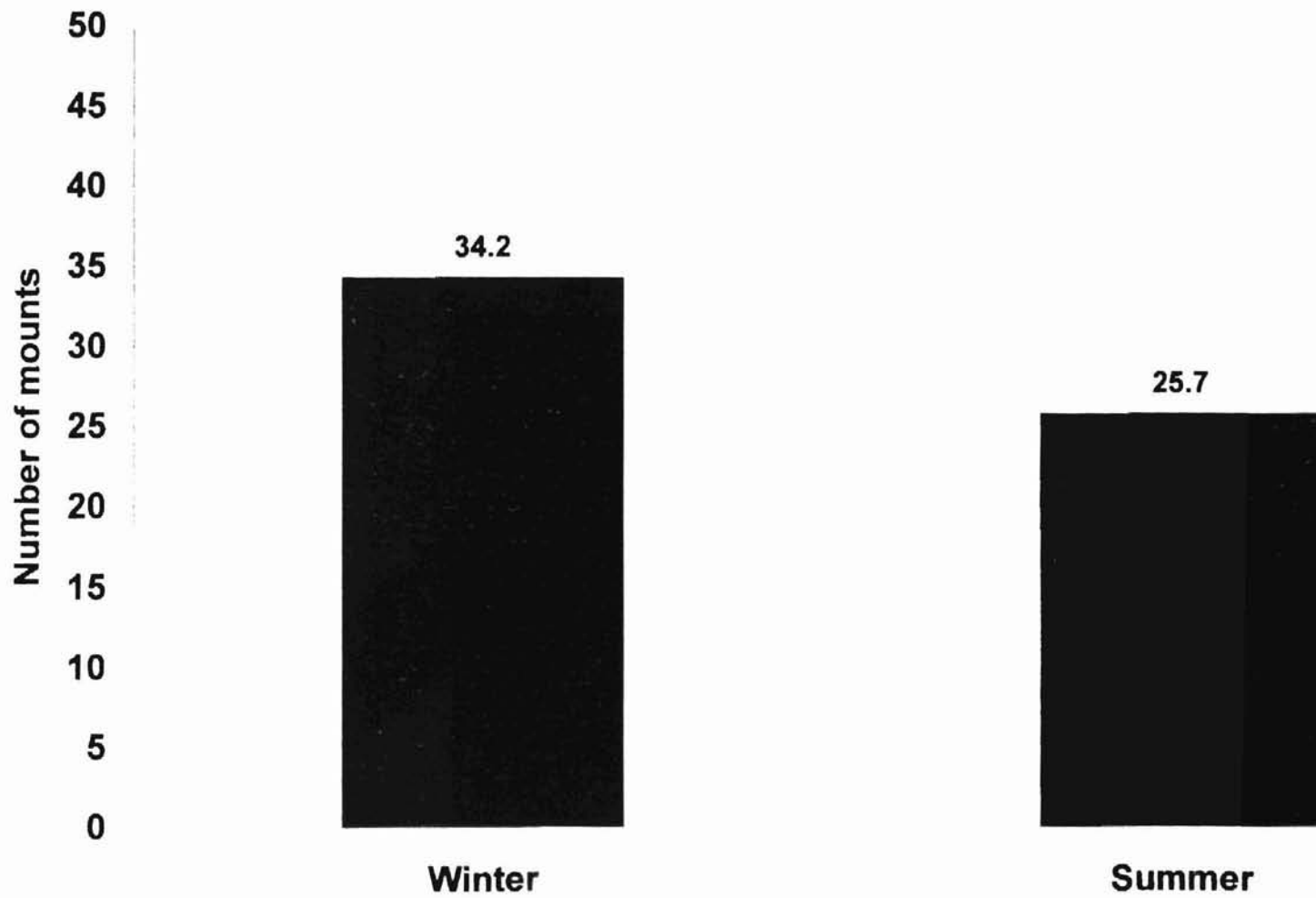


Figure 6. Influence of season on the number of times estrous cows are mounted (P < 0.06).

increased ( $P < 0.05$ ;  $11.6 \pm 1.5$  h vs  $16.9 \pm 0.9$  h, Figure 7). When 2 or 3 cows were in estrus, the duration of estrus was intermediate in length and not different from one cow in estrus or 4 to 6 cows were in estrus. When seven or more cows were in estrus the duration of estrus was not altered compared with when 4 to 6 cows were in estrus. Almost three times as many estruses were less than 8 h in duration when only one cow was estrus, compared with when two or more cows were estrus (Figure 8). Over 87% of estruses were longer than 12 h, and over 62% were longer than 16 h, when seven or more cows were in estrus. During both seasons the duration of estrus ranged from .5 h to 28.2 h when only one cow was in estrus.

Duration of estrus was longer ( $P < 0.01$ ) in winter ( $16.8 \pm 0.7$  h) than in summer ( $13.9 \pm 0.8$  h; Figure 9) and shorter ( $P < 0.05$ ) when cows were on pasture ( $14.2 \pm 0.7$  h) than in drylot ( $16.4 \pm 0.8$  h; Figure 10). The number of mounts per estrous was correlated ( $P < 0.0001$ ) with the duration of estrus ( $r = 0.51$ ).

Duration of the longest interval between mounts in an estrus decreased ( $P < 0.002$ ) when the number of cows in estrus at one time increased from one ( $5.3 \pm 0.7$  h) to seven or more ( $2.6 \pm 0.3$  h; figure 11). The longest interval between mounts was not decreased ( $P > 0.10$ ) when two to six cows were estrus simultaneously, compared with one cow in estrus. The duration of the longest interval between mounts was not influenced by season, confinement area or interactions between the main effects. The longest interval between mounts occurred more frequently during the second half of estrus (63.1% of the time). The longest interval between mounts during the second half of estrus ( $3.9 \pm 0.2$  h) was longer ( $P < 0.05$ ) than the longest interval between mounts in the first half of estrus ( $3.1 \pm 0.3$  h). Duration of the longest interval between mounts for an estrus was 11.1 h when only one cow was in estrus. The number of cows in estrus did

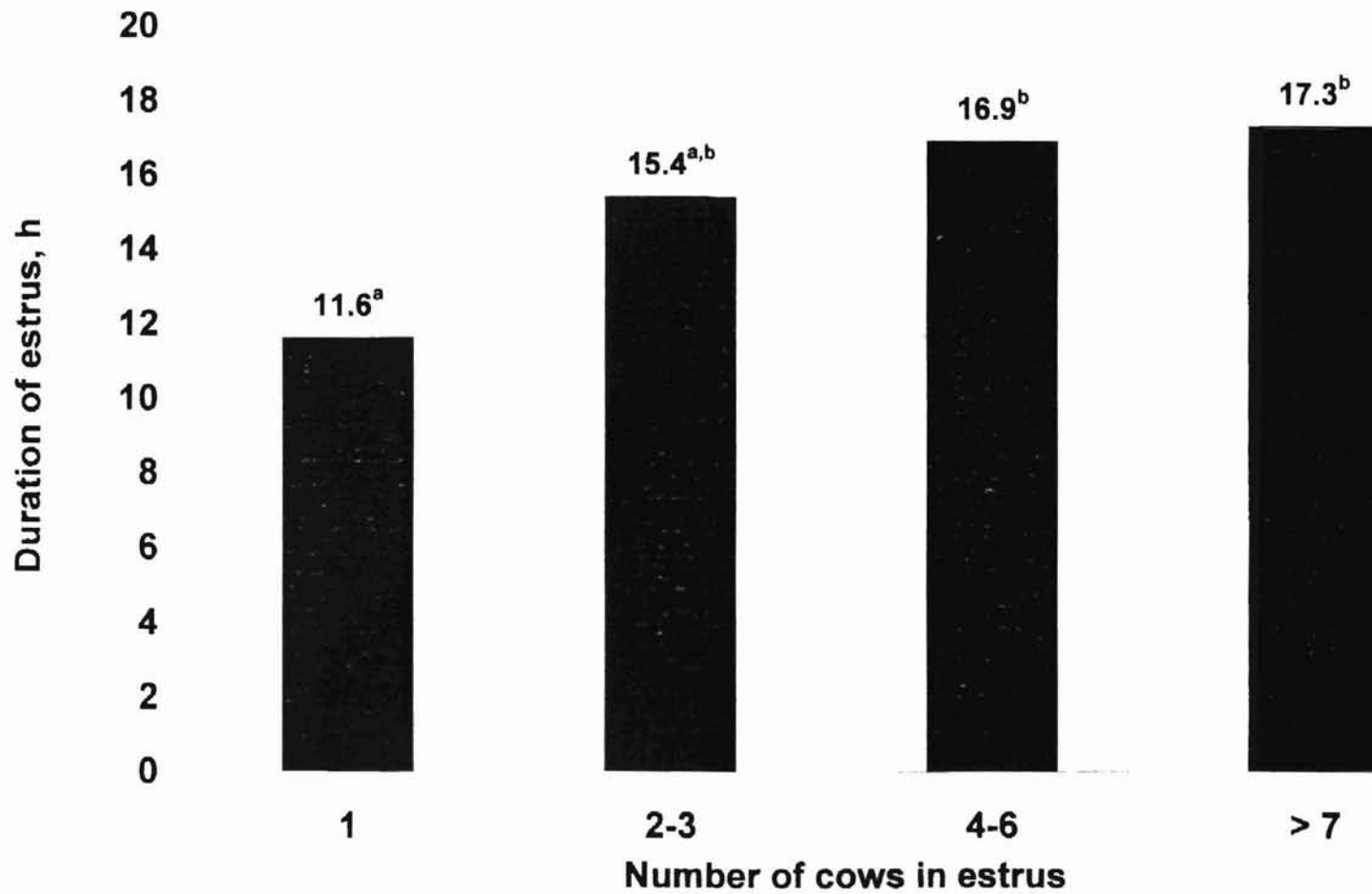


Figure 7. Influence of the number of cows in estrus on the duration of estrus ( $P < 0.01$ ).

<sup>a,b</sup> differ  $P < 0.05$ .



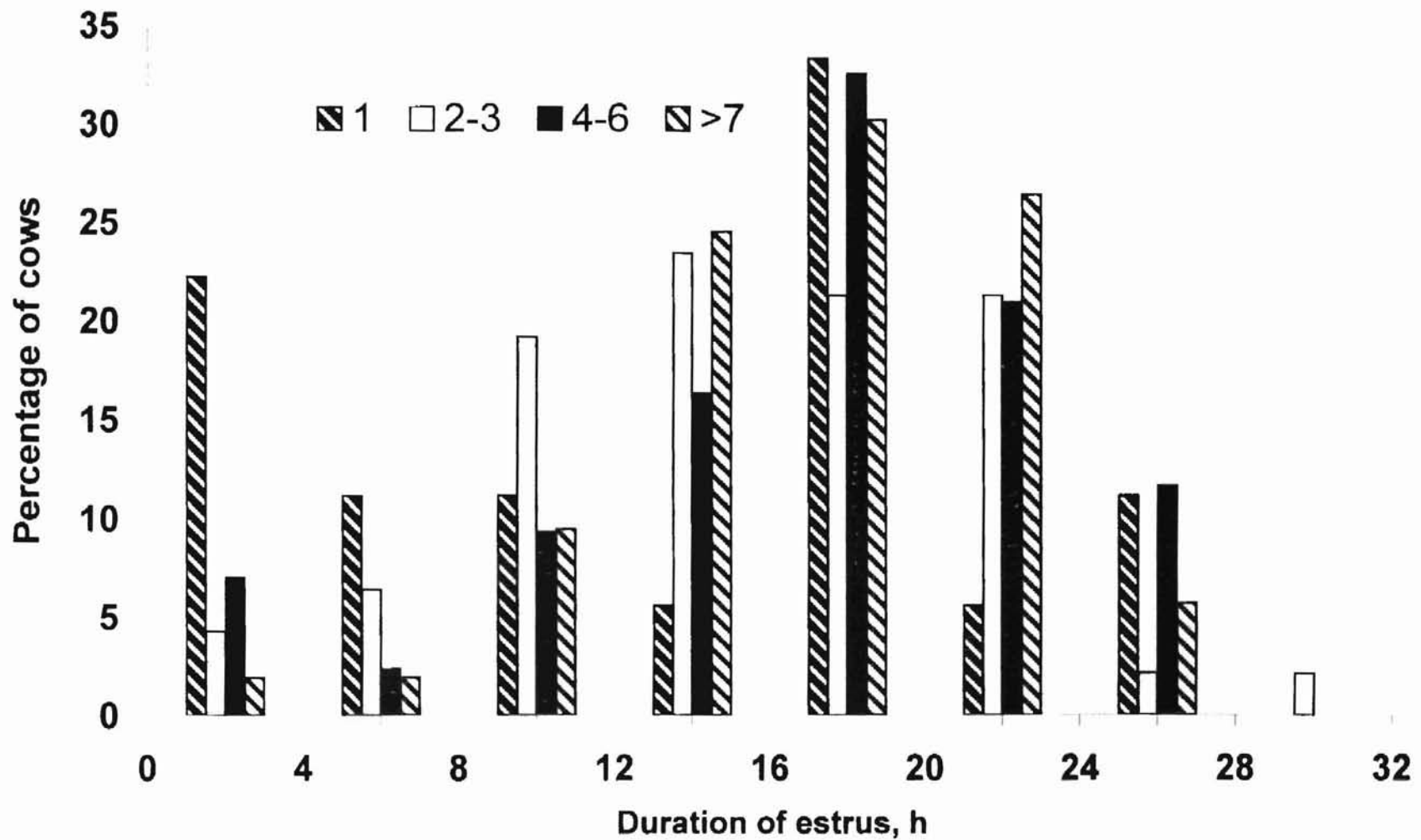


Figure 8. Influence of the number of cows in estrus on the percentage of cows with different durations of estrus.

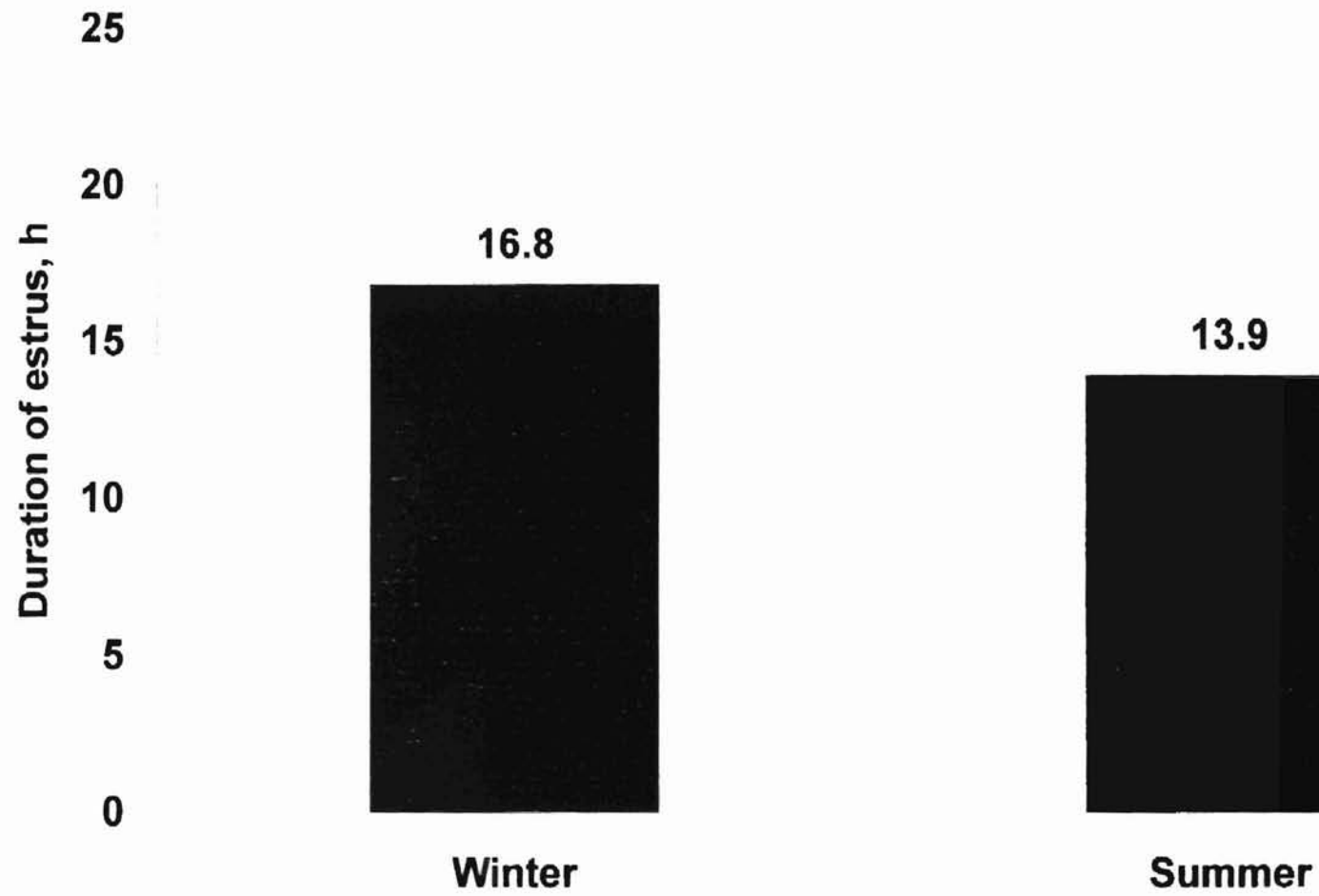


Figure 9. Influence of season on the duration of estrus ( $P < 0.01$ ).

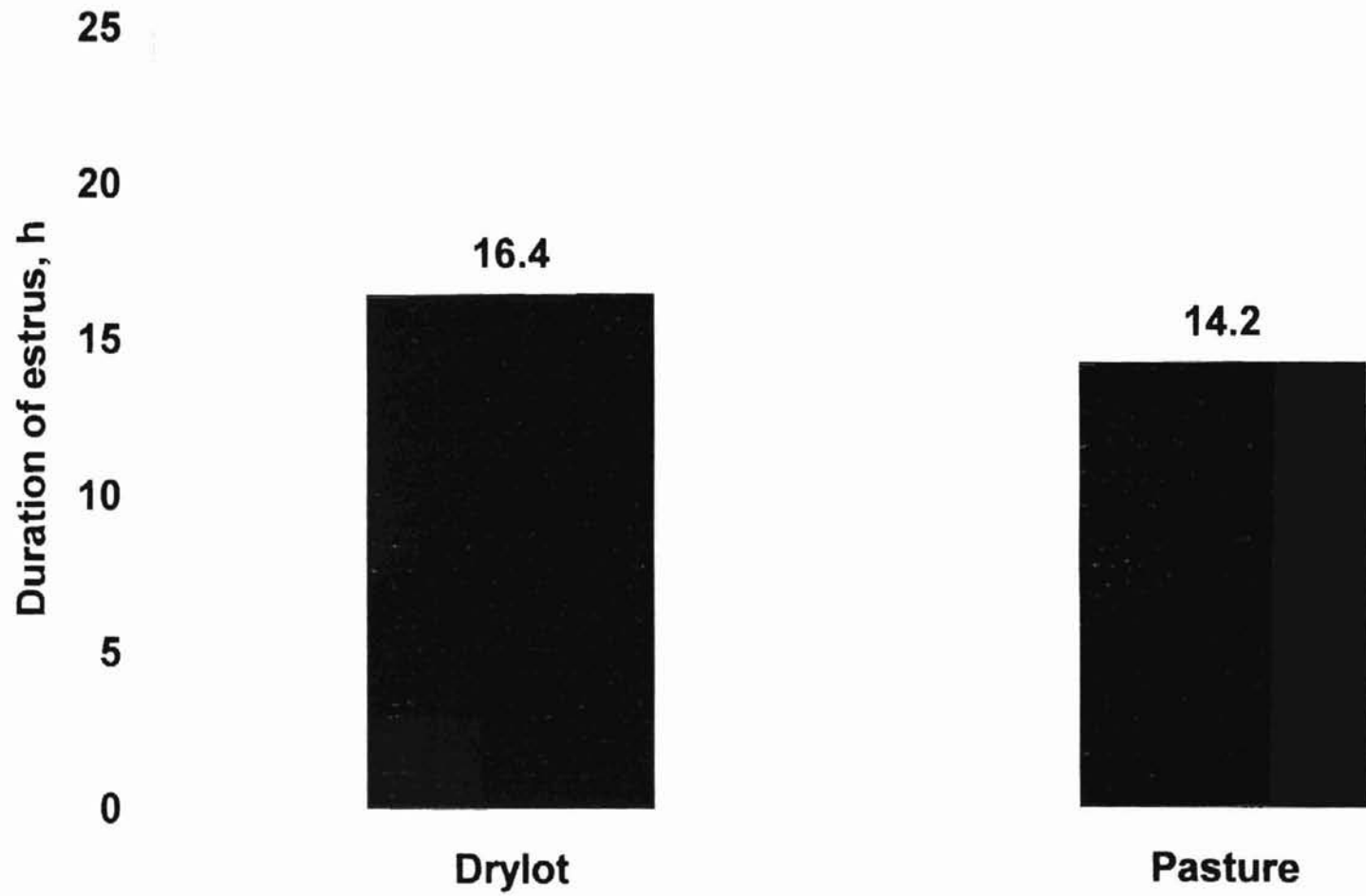


Figure 10. Influence of confinement area on the duration of estrus ( $P < 0.05$ ).

not influence ( $P > 0.10$ ) whether the longest interval between mounts was in the first or second half of estrus.

## Discussion

HeatWatch<sup>®</sup> detected all estruses that were recorded with constant observation of cows during 28 h. HeatWatch<sup>®</sup> records mounts in which the pressure sensor is activated for 2 or more seconds. In some cases, the positioning of cows may have prevented activation of the sensor, which could contribute to more visually detected mounts than mounts recorded by HeatWatch<sup>®</sup>. When cows were mounted simultaneously, HeatWatch<sup>®</sup> recorded the concurrent mounts. During the visual observation period the sexually active group was readily identifiable, especially at night when other cows were resting, and mounting rarely occurred outside of the active group. Similar observations on behavior of sexually active groups have been made by Williamson and coworkers, (1972b). Others have found that HeatWatch<sup>®</sup> has an efficiency of 89.5 – 100% and accuracy of 78 - 100% (Stevenson et al., 1996b; Timms et al., 1997; Xu et al., 1998).

Ninety-two percent of cows responded to the initial synchronization with estrous behavior and normal concentrations of progesterone in plasma. This is a greater percentage than in previous studies when beef and dairy, cows and heifers (Britt et al., 1978; Lauderdale and Sokoloski, 1979; Nkuuhe and Manns, 1985) were synchronized with two PGF<sub>2α</sub> treatments 11 to 12 days apart. All cows had normal estrous cycles at the beginning of this study, which probably increased the response rate to the initial synchronization. In addition, fewer estruses were probably undetected because cows were monitored continuously with HeatWatch<sup>®</sup>.

Luteal regression occurred in 95% of cows with a functional CL when treated with PGF<sub>2α</sub> between days 6 and 18 of the cycle and in 90% of the cows this was accompanied with formation of a new CL. Ninety-five percent of cows that had luteal regression after treatment with PGF<sub>2α</sub> exhibited estrous behavior. Eighty-one percent of cows that had a functional CL when treated with PGF<sub>2α</sub> on d 6 to 18 of the cycle responded with both estrous behavior and normal concentrations of progesterone in plasma. A similar response was observed in Holstein heifers (Stevenson et al., 1984; Watts and Fuquay, 1985), but fewer dairy cows and heifers exhibited estrus and ovulated when treated with PGF<sub>2α</sub> every 11 d for 55 to 66 days without regard to luteal status at treatment (Castellanos et al., 1997). Ovulation without estrus occurred 4.5% of the time in this study, which is less than previously reported (Plasse et al., 1970; Orihuela et al., 1983; Rodtian et al., 1996). The use of HeatWatch<sup>®</sup> probably resulted in fewer unobserved estruses in this study.

Cows in the drylot were in estrus sooner after PGF<sub>2α</sub> treatment than cows on pasture (61.8 ± 3.1 h vs 72.8 ± 3.3 h). This suggests that the interaction of cows in a smaller area hastens the onset of estrus. The two hour longer duration of estrus for cows in drylot than for cows on pasture would account for only a small part of this variation. The causes of shorter intervals to estrus after PGF<sub>2α</sub> in the drylot are not fully understood but may be related to the closer association of cows in drylot than on pasture.

The interval to estrus was 14 h longer in cows treated with PGF<sub>2α</sub> between d 10 to 13 of the cycle (76.7 ± 3.7 h) than for cows treated on d 6 to 9 or d 14 to 18 of the cycle (62.3 ± 4.7 and 62.9 ± 3.6 h, respectively). Similarly, Tanabe and Hann, (1984) and Watts and Fuquay, (1985) found a longer interval from PGF<sub>2α</sub> to estrus when dairy

heifers were treated with PGF<sub>2α</sub> on d 8 to 11 of the cycle than when treated on d 5 to 7 of the cycle. Comparable to the short interval to estrus when beef cows were treated with PGF<sub>2α</sub> on d 6 to 9 of the cycle in the present study, dairy heifers treated on d 5 to 7 of the cycle (Johnson, 1978; Watts and Fuquay, 1985), and beef cows treated on d 5 to 9 of the estrous cycle (King et al., 1982) commenced estrus in 57 to 60 h after treatment. However, Stevenson et al., (1984) and Tanabe and Hann, (1984) found shorter intervals to estrus in Holstein heifers after treatment with PGF<sub>2α</sub> on d 5 to 8 of the cycle, (44 to 50 h). The interval to estrus was  $76.7 \pm 3.7$  h when we treated beef cows on d 10 to 13 of the cycle. This is slightly longer than the interval to estrus in dairy heifers treated on d 8 to 11 of the cycle (70 to 72 h; Tanabe and Hann, 1984; Watts and Fuquay, 1985), and in beef cows treated on d 10 to 15 of the cycle (67 h; King et al., 1982). Similar to the interval to estrus of  $62.9 \pm 3.6$  h when cows were treated on d 14 to 18 of the cycle in the current study, Stevenson et al., (1984) found a 61 h interval to estrus when Holstein heifers were treated with PGF<sub>2α</sub> on d 14 to 16 of the cycle. Differences in intervals to estrus between studies may be influenced by the methods of estrous detection and management conditions. The interval between PGF<sub>2α</sub> treatment and estrus was not affected by breed (King et al., 1982). In agreement with our results, the number of cows in estrus at the same time did not influence the duration from treatment with PGF<sub>2α</sub> to estrus (Walton, et al., 1987).

Percentage of beef cows with luteal regression and estrus was similar when PGF<sub>2α</sub> was given during early, mid, or late cycle. Similarly, Stevenson et al., (1984) and Tanabe and Hann, (1984) found that treatment with PGF<sub>2α</sub> on or after d 5 and before d 16 of the cycle, did not influence the percentage of dairy heifers with luteal regression. However,

Watts and Fuquay, (1985) found an increase in the response to  $\text{PGF}_{2\alpha}$  in dairy heifers as day of the cycle at treatment increased from d 5 to 7 to d 12 to 15. This may be related to luteal regression in only 10% of heifers treated with  $\text{PGF}_{2\alpha}$  on d 5 of the cycle.

A greater percentage of cows treated with  $\text{PGF}_{2\alpha}$  on d 6 to 9 (75 %) and 14 to 18 (53 %) exhibited estrus in a 20 h period (40 to 60 h after treatment) than those treated on d 10 to 13 (34%) of the estrous cycle. Similarly, more dairy heifers treated with  $\text{PGF}_{2\alpha}$  on d 7 and d 15 of the cycle were in estrus during a 24 h period than when heifers were treated on d 11 (Tanabe and Hann, 1984).

Confinement of cows to drylot or pasture did not influence the number of mounts per estrus. Footing surface in both drylot and pasture were conducive to mounting and both areas were large enough to prevent crowding. Zebu cows penned and fed hay at night and allowed to graze during the day, exhibited more mounts in a pen than when on pasture (Mattoni et al., 1988).

Duration of estrus was 2 h longer in the drylot than on pasture, but the number of times a cows was mounted was not influenced by drylot or pasture. Therefore, estrus should be efficiently detected on pasture or in drylot.

Length of estrus ( $16.8 \pm 0.7$  in winter, and  $13.9 \pm 0.8$  in summer) was similar to reports for synchronized bos indicus cows (Lemaster et al., 1999), Charolais cows (Galina et al., 1982), dairy heifers (Hurley et al., 1982), and zebu cows (Orihuela et al., 1983), but longer than for synchronized beef heifers (Stevenson et al., 1996b; Rae et al., 1999), postpartum beef cows (Hurnik and King, 1987; Ciccioili et al., 2000; Lents, 2001) and lactating dairy cows (Walton et al., 1987; Walker et al., 1996; Dransfield et al., 1998; Xu et al., 1998).

Duration of estrus was approximately 3 h shorter in summer than winter.

However, we previously found duration of estrus was two hours longer in the summer than in the winter (White et al., 2002). Similar to the result of this study, Abilay et al., (1975) found a decrease ( $P < 0.05$ ) in the duration of estrus when dairy heifers were exposed to 33.5 C compared with exposure to 18.2 C. Yelich et al., (1999) also found shorter synchronized estruses in summer than in winter, but season did not influence spontaneous estruses. Contrary to these reports, Pennington et al., (1985) found a longer duration of estrus in dairy cows in hot weather than in cold weather in a temperate climate. The degree to which climatic conditions influence mounting behavior and the duration of estrus would be affected by the severity of ambient temperature, air movement, geographical location and other factors.

The longest interval between mounts decreased as the number of cows in estrus increased. Although no seasonal effect on the longest interval was found in this study, we previously found longer ( $P < 0.05$ ) intervals between mounts in summer than in winter (White et al., 2002). In that study, four or fewer cows were in estrus, simultaneously, and the effect of the number of cows in estrus was not evaluated.

This is the first study to demonstrate that increasing the number of beef cows in estrus has a dramatic effect on the number of times a cow is mounted during estrus and the duration of estrus. Similarly, increasing the number of dairy cows in estrus increases the duration and number of mounts per estrus (Hurnik et al., 1975; Helmer and Britt, 1985; Pennington et al., 1985; Walton et al., 1987; Van Vliet and Van Eerdenburg, 1996). Dairy cows in a free stall area exhibited an average of 11.2 mounts per estrus during 7.5 h when only one cow was estrus, which increased to 52.6 mounts during 10.1 h when three cows were in estrus at the same time (Hurnik et al., 1975). Similarly, in a free stall



facility with slatted flooring, the number of mounts per dairy cow increased from an average of 3.5 mounts in 2.5 h with only one estrous cow to 40 mounts in 17 h with four cows in estrus at the same time (Walton et al., 1987). Mounts per cow increased by 9.9 and duration of standing estrus increased by 3.7 h for every additional dairy cow in estrus, simultaneously, from one to six cows (Pennington et al., 1985). In a group of nine dairy heifers the number of mounts increased ( $P < 0.05$ ) as the number of heifers in estrus at the same time increased from 1 (4.5 mounts per estrus) to 4 (39.0 mounts per estrus; Helmer and Britt, 1985).

The number of mounts per estrus and the duration of estrus are both affected by days postpartum. Only 19% of first postpartum ovulations in dairy cows were detected visually which increased to 37% and 79% in second and third postpartum ovulations, respectively (Peter and Bosu, 1986). We previously found postpartum beef cows received only 6 to 13 mounts in 4 to 6 h at the first postpartum estrus (Ciccioli and Wettemann, 2000; Lents, 2001). Increasing the number of cows in estrus could allow more cows to be detected in estrus when cows are estrus for a short duration with few mounts or when there are long intervals between mounts.

### Implications

Our results indicate that detecting a single cow in estrus can be very difficult. Increased visual observation, especially during summer, may be needed to detect estrus when only one cow is in estrus. Synchronization of estrus with more cows in estrus at the same time, increased the number of times estrus cows were mounted and the duration of estrus, and decreased the longest interval between mounts. This greater display of estrus could allow fewer visual observations to detect estrous cows than necessary when only

one cow is in estrus. The number of days that observations must be made is also decreased if cows estrous cycles are synchronized. Cows were in estrus longer in the drylot than in the pasture but the number of mounts per estrus was not different between drylot and pasture. Therefore, visual estrus detection of cows in a drylot or pasture should be equally successful.

## CHAPTER IV

### SUMMARY AND CONCLUSIONS

Cows in a drylot exhibit estrus sooner than cows in pasture after treatment with PGF<sub>2α</sub>. Cows treated on d 6 to 9 and 14 to 18 of the estrous cycle have a shorter interval to estrus after PGF<sub>2α</sub> treatment and greater synchrony of estrus than cows treated on d 10 to 13 of the cycle. Cows with a greater synchrony of estrus could require a shorter period of observation to detect estrus.

Increasing the number of cows in estrus at the same time increases the duration and the number of mounts per estrus and decreases the longest interval between mounts. Producers typically observe cattle once in the morning and once in the evening to detect cows in estrus. A longer duration of estrus increases the chance that an estrous cow will be detected during one or more observation periods. Likewise, increasing the number of mounts per estrus and decreasing intervals between mounts increases the chance that an estrous cow will be mounted during an observation period. Synchronizing estrus in cows will decrease the number of days required to detect estrus and decrease the duration of observation needed during that period to detect a large percentage of cows in estrus. The use of estrous detection aids such as tail chalking and mount detectors could also decrease the number of observations needed. Having a modified bull for estrous detection in a group of estrous synchronized cows may not be necessary since other estrous cows efficiently detect estrus.

Since the number of mounts per estrus is fewer and duration of estrus is shorter in summer than in winter, the number of observation periods in a day may need to be increased for estrous detection during the summer. It is difficult to detect estrus in postpartum beef cows, which are in estrus for a short time and are only mounted a few times. Synchronization of estrus in postpartum cows could increase the number of estrous cows that are detected. Although synchronization of cows increases the labor involved to treat cows, it decreases the total number of days needed for estrous detection for AI and concentrates labor.

Cows in drylot were in estrus 2 h longer than cows on pasture, but the number of mounts per estrus was not different between drylot and pasture. Therefore, the ability to detect estrus should be similar for cows in drylot and a small pasture. However, for actual identification of cows in estrus, it might be advantageous to have cows in a smaller confinement area.

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