Suckled cows and virgin heifers received a novel treatment that included PGF$_{2\alpha}$, GnRH, and norgestomet, with the objective of inducing estrus in prepubertal heifers and anestrous suckled cows, as well as synchronizing ovulation in estrus-cycling females. The treatment consisted of two injections of PGF$_{2\alpha}$ (day 14 and 0) plus 100 $\mu$g of GnRH and a 6-mg norgestomet ear implant on day 7. The implant was removed 24 h after the second injection of PGF$_{2\alpha}$ (day 0), and a second injection of GnRH was given 30 hours after implant removal. The treated females were inseminated 18 hours after the second injection of GnRH or at estrus, if it was detected before the second GnRH injection. Pregnancy rate in the treated females was greater than in control females that had received two injections of PGF$_{2\alpha}$ 14 days apart and were inseminated at estrus or at one fixed time (60.2 vs. 48%). The treatment successfully induced a fertile ovulation in previously prepubertal heifers and anestrous cows, resulting in 63.5% pregnancies vs. 26.5% for controls. In addition, in females not showing estrus, the treatment increased pregnancy rate following a fixed-time insemination (treatment vs. control; 60.0 vs. 3.8%). We concluded that treatment with PGF$_{2\alpha}$, GnRH, and norgestomet induced estrus and increased pregnancy rates in prepubertal heifers, anestrous cows, and cycling females.

(Key Words: GnRH, Norgestomet, PGF$_{2\alpha}$, Heat Synchronization, Prepubertal Heifers, Anestrous Suckled Cows.)

Estrus-synchronization programs improve reproductive efficiency by reducing the length of breeding and calving seasons, increasing calf weaning weights, and grouping cows and heifers so artificial insemination (AI) can be used more efficiently. They are not designed to induce estrus in prepubertal heifers or anestrous suckled beef cows. Treatments involving single or multiple injections of gonadotropin-releasing hormone (GnRH) given 10 to 12 days apart and(or) implants of norgestomet have been used to jump start (induce estrus) noncycling heifers and cows. The result of injecting GnRH is to induce secretion of LH and FSH and cause ovulation of mature follicles. Norgestomet primes the hypothalamic-pituitary axis for release of the endogenous GnRH, LH, and FSH necessary for follicle growth. In prepubertal heifers and anestrous suckled cows, the norgestomet implant also prevents the short luteal phase or short estrous cycle that normally follows the first pubertal or postpartum ovulation. That short cycle prevents the continuation of pregnancy, even when fertilization occurs. Therefore, our objective was to test the effect of this novel treatment using prostaglandin F2 $\alpha$ (PGF$_{2\alpha}$), GnRH, and norgestomet for its ability to induce estrus and increase conception in prepubertal heifers and anestrous suckled cows, as well to synchronize estrus in cycling females before one fixed-time insemination.

In a 2-year study, purebred Angus, Hereford, and Simmental heifers and suckled cows were assigned to two treatments: 1) two injections of PGF$_{2\alpha}$ 14 days apart (control); or 2) two injections of PGF$_{2\alpha}$ (days 14 and 0) plus 100 $\mu$g of GnRH and a 6-mg norgestomet implant.
implant on day 7 (ovulation synchronization; Figure 1). The norgestomet implant was re-
moved 24 h after the second injection of PGF$_{2\alpha}$ (day 0). A second injection of 100 µg of GnRH was given 30 h after implant removal. Three blood samples were collect ed (24, 14, and 7 days) before the first GnRH injection to deter-
mine estrus-cycling status.

Control females were inseminated 12 to 16 h (AM-PM rule) after first detected estrus until 80 h after the second PGF$_{2\alpha}$ injection, when all remaining females were inseminated. The females in the ovulation synchronization treatment were inseminated either at estrus or at 18 h after the second injection of GnRH (48 h after implant removal or 72 h after the second PGF$_{2\alpha}$ injection). Pregnancy status was determined by intrarectal ultrasonography on days 34 or 35 after insemination.

**Results and Discussion**

The majority of heifers (87%) and cows (66%) were cycling at the time the treatments were initiated, based on serum progesterone concentrations 24, 14, and 7 days before the time of the GnRH injection and implant (Figure 1). Similar proportions of noncycling control and ovulation-synchronized heifers showed estrus and were inseminated at estrus (46.9 vs. 34.6%). For noncycling cows, more (P<.05) control than ovulation- synchronized cows were inseminated at estrus (80 vs. 39.5%). The remaining heifers and cows were inseminated at a fixed time after PGF$_{2\alpha}$.

No differences in pregnancy rates were detected among breeds or parity groups (heifers and cows). Pregnancy rate was greater (P<.01) in the ovulation-synchronized females than in controls (60.2 vs. 48%). Control and treated females that were already cycling had similar pregnancy rates (56.2 vs. 58.8%). The experimental treatment increased pregnancy rates in noncycling females (26.5 vs. 63.5%; Table 1). Our results indicate that the ovulation synchronization treatment successfully induced a fertile ovulation in previously prepubertal heifers and anestrous suckled cows.

Pregnancy rates were similar in ovulation-synchronized females (66.1 vs. 60.6%), regardless of whether inseminated at estrus or at one fixed time (72 hours after PGF$_{2\alpha}$; Table 2). In contrast, controls inseminated at estrus had greater pregnancy rates than controls inseminated at one fixed time (80 hours after PGF$_{2\alpha}$), 60 vs. 3.8%.

These results demonstrate that our novel ovulation synchronization treatment induced a fertile ovulation in both noncycling and cycling heifers and cows. Furthermore, conception rate by fixed timed insemination after the ovulation synchronization equalled that achieved when inseminations were made at estrus in controls. Therefore, our treatment synchronizes ovulation with estrus. We conclude that treatment with PGF$_{2\alpha}$, GnRH, and norgestomet induced estrus and increased pregnancy rates in prepubertal heifers, anestrous cows, and cycling females. In addition, the treatment increased pregnancy rates following one fixed-time insemination.
Ovulation Synchronization

A = 25 mg of Lutalyse® (PGF₂₀α)
B = 100 g of Cystorelin® (GnRH)+ 6-mg ear implant of norgestomet
C = 25 mg of Lutalyse
D = Removal of implant
E = 100 g of Cystorelin
F = Insemination

Control

A = 25 mg of Lutalyse
B = 25 mg of Lutalyse
C = Insemination (in absence of detected estrus)

Figure 1. Treatment Protocols for Ovulation Synchronization Treatment and Control
Table 1. Pregnancy Rates in Previously Noncycling and Estrus-Cycling Heifers and Suckled Cows after Synchronized Ovulation using PGF\textsubscript{2\alpha}, GnRH, and Norgestomet

<table>
<thead>
<tr>
<th>Parity</th>
<th>Control No.</th>
<th>% Pregnant</th>
<th>Ovulation Synchronized No.</th>
<th>% Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncycling Heifers</td>
<td>49</td>
<td>26.5\textsuperscript{a}</td>
<td>52</td>
<td>63.5\textsuperscript{a}</td>
</tr>
<tr>
<td>Cows</td>
<td>45</td>
<td>28.9</td>
<td>47</td>
<td>59.6</td>
</tr>
<tr>
<td>Cycling Heifers</td>
<td>130</td>
<td>56.2\textsuperscript{c}</td>
<td>119</td>
<td>58.8\textsuperscript{a}</td>
</tr>
<tr>
<td>Cows</td>
<td>32</td>
<td>56.3</td>
<td>87</td>
<td>58.6</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Heifers and suckled cows were classified as noncycling or estrus-cycling based on serum concentrations of progesterone measured in three samples (24, 14, and 7 days before the second injection of PGF\textsubscript{2\alpha}).

\textsuperscript{b}See Figure 1 for details.

\textsuperscript{c}Interaction (P=.08) of treatment and estrus-cycling status.

Table 2. Effect of Fixed-Time Insemination on Pregnancy Rates in Heifers and Suckled Cows

<table>
<thead>
<tr>
<th>AI Time\textsuperscript{b}</th>
<th>Control No.</th>
<th>% Pregnant</th>
<th>Ovulation Synchronized No.</th>
<th>% Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrus</td>
<td>127</td>
<td>66.1\textsuperscript{a}</td>
<td>66</td>
<td>60.6\textsuperscript{a}</td>
</tr>
<tr>
<td>Fixed time</td>
<td>52</td>
<td>3.8\textsuperscript{y}</td>
<td>91</td>
<td>60.0\textsuperscript{a}</td>
</tr>
<tr>
<td>Total</td>
<td>179</td>
<td>48.0</td>
<td>171</td>
<td>60.2</td>
</tr>
</tbody>
</table>

\textsuperscript{a}See Figure 1 for details.

\textsuperscript{b}Controls were inseminated at one fixed time (80 hours after the second injection of PGF\textsubscript{2\alpha}) in the absence of estrus. Ovulation synchronized females were inseminated 18 hours after the second GnRH injection (48 hours after the norgestomet implant was removed or 72 hours after the second injection of PGF\textsubscript{2\alpha}) in the absence of estrus.

\textsuperscript{c}Interaction (P<.01) of treatment and insemination time.