Effects of Dalmazin on Luteal Cysts in Crossbreed Cows in Multan, Punjab (Pakistan)

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ABSTRACT

This study was conducted on the crossbreed cows (Sahiwal x Friesian) n=323 maintained at the Multan Dairy Farm Vehari Road Multan. The study aims to assess the response of treatment of crossbreed cows identified to possess luteal cyst using the prostaglandin analogue. The animals which did not show estrus up to 80-100 days post partum were considered as cystic disorder. These animals were initially examined through rectal palpation. Sixty five (20.12%) animal showed no visible estrus signs. The incidence of postpartum disorders were luteal cyst, 15 (4.64%), follicular cyst, 11 (3.40%), corpus luteum 12 (3.71%), persistent corpus luteum 12 (3.71%), Anoestrous 13 (4.02%), and cystic ovary 2 (0.61%) through rectal palpation in the crossbreed cows. Three clinical examinations with a 7 day interval were performed in these cows and blood samples were also collected for progesterone analysis through radioimmunoassay (RIA). The results of the rectal palpation were confirmed by plasma progesterone concentration. The concentration of the progesterone before treatment in the blood was 1.44±0.44 ng/ml, 1.12±0.56 ng/ml and 0.56±0.23 ng/ml during 1st, 2nd and 3rd week respectively. The 8 cows were found as having luteal cyst. The treatment with Dalmazin (PGF2α) changed the plasma concentration of progesterone and it was 1.25±0.47 ng/ml, 3.88±0.88 ng/ml and 6.03±1.91 ng/ml during 1st, 2nd and 3rd week respectively. All the treated animals were responded, 75% were pregnant and 25% were in regular cycling. The dalmazin (PGF2α) treatment enhanced the reproductive efficiency of the crossbreed cows, which were suffering from luteal cyst.

Key words: Luteal Cysts, Dalmazin, Progesterone, Estrogen, GnRH, RIAS.

INTRODUCTION

The estrus duration of 6 to 24 h with a mean of 15 h in the cattle is designated as d 0, or the start of the cycle. The predominant reproductive hormone during the estrus phase of the cycle is estrogen (Hansel & Echternkamp, 1972). In cows, the estrus period reoccurs after every 21 day but it may range from 17 to 24 days and also this cycle may fluctuate between periods of estrogens (E) to progesterone (P4) dominance. The preparation of uterus and potential conceptus is predominated by progesterone (P4) during the luteal phase. The increase in milk yield for lactating dairy cows is associated with a decrease in fertility (Khan, et al., 2012), concluded that conception rate increase by 16.6% through hormonal therapy followed by fixed time AI in Kundi buffalo under field conditions.

In the normal human female the corpus luteum (CL) grows to approximately 1.5 cm in diameter, reaching as stage of development approximately 7 or 8 days after ovulation (Guyton & Hall, 2000). In the cow the growth is completed by days 11-13 of the cycle and mature corpus luteum may have a diameter from 22 mm–30 mm depending on the shapes of the corpus luteum. (Salisbury et al.,1985).

A mean time to estrus of 70 and 62 hours respectively was resulted through prostaglandin administration in mid-cycle (day 8 to day 11) and later in the luteal phase (day 12 to 15) (Stevenson et al., 1984). Ovarian cysts are an-ovulatory structures and as long as they persist, cows will remain infertile (Youngquist & Threlfall, 2007). In some studies, GnRH plus PGF2α was found highly effective in expelling uterine fluid, resolving ovarian cyst and achieving considerable rate of conception (Shah, 2009).

The following objectives were determined to accomplish our purpose:

1. To study the estrous cycle of cattle
2. The prevalence incidence of the luteal cyst in the cross breed cows
3. The accuracy of rectal palpation for the diagnosis of luteal cyst

MATERIALS AND METHODS

The present study was carried out on the crossbreed cows (Sahiwal x Friesian) from a dairy herd at the Multan Dairy Farm Vehari Road Multan. Cross breed cows were housed in a tie stall barns and nourish green fodder, cotton seed cake and
wheat barn hey and concentrate. The cows after 80-
100 days post partum were examined through rectal
palpation to check the luteal cysts. These conditions
were confirmed by progesterone profile in the blood
plasma using RIAs.

Rectal Palpation

Procedure
1) The cow was restrained by placing it in a
tube and scaffold.
2) The cow can be gently touched on the flank
or back to make it aware of the palpator’s
presence and to allow evaluation of the
cow’s temperament.
3) The disposable palpation sleeve with a
tighter-fitting latex glove fitted over the hand
was used.
4) The commercially available lubricants were
used to lubricate the gloves.
5) By grabbing the tail with the ungloved hand,
the gloved hand is inserted into the rectum
by joining the fingers and thumb together
(cone shape).
6) If there is abundance of feces, it can be
removed by the palpator, otherwise the
rapid motions are avoided which can cause
intake of air in rectum and also shifting
rectum balloon outward.

Collection of Blood Sample

Disposable syringes were used to collect
blood samples from jugular vein for three weeks from
the post partum animals for the analysis of the
plasma progesterone level after regular interval of
one weak. These blood samplings were also
repeated after the treatment of Dalmazin (1ml).
Heparin was used as an anticoagulant. 1 ml of
heparin was mixed in 100 ml of 1% saline solution.
In each test tube mixed 1 ml of heparin solution and
5 ml of blood sample. Then these blood samples
were centrifuged for 10-15 minutes at 3000rpm. The
serum was harvested with the help of micropipette.
The serum was analyzed by radio immune assay
(RIAs).

Progesterone Assays

Procedure
By using RIAs, progesterone concentration
in blood sample was determined as described by
Hoffmann. At a room temperature (25°C) all
reagents were mixed thoroughly by gentle inversion
before use. In appropriate tubes with pipette 25µl of
the standards controls and 500µl of progesterone (I-
125) reagent were added. After shaking the test
tubes gently by hand, the tubes were incubated in a
water bath at 37±2°C for 60-70 minutes.

Except total count tubes, decant all tubes and
simultaneous inversion with a sponge rack in to
a radioactive waste receptacle. For complete
drainage, stroke the tubes sharply on absorbent
material for a minimum of 2 minutes. Furthermore,
for one minute count all tubes in gamma counter and
then dispose off all non-radioactive and non-
hazardous reagents by flushing with large amounts
of water to prevent buildup of chemical hazards in
plumbing system.

Statistical Analysis

SPSS was used for the calculation of
means (standard descriptive statistics) and the
results were described as mean ± SEM and
percentage. Furthermore, one way ANOVA by using
SPSS was performed to compare the different type
of means from various parameters. In this test
P<0.05 was considered as statistically significant.

RESULTS

The results of the 323 crossbreed cows
were investigated, 65 (20.12%) failed to show
visible sign of estrous up to 90 days post partum.
The incidence of post partum disorders were based
on rectal palpation such as the luteal cyst 15
(4.64%), anoestrous 13 (4.02%), corpus luteum 12
(3.71%), persistent corpus luteum 12 (3.71%),
follicular cyst, 11 (3.40%), and cystic ovary 2
(0.61%) (Fig., 1).

Fig., 2 explained the plasma progesterone
profile in the blood before treatment. Blood
samples of each cow were taken for three consecutive
weeks. The concentration of the progesterone in the
blood was 1.44±0.44 ng/ml, 1.12±0.56 ng/ml and
0.56±0.23 ng/ml during 1st, 2nd and 3rd week
respectively.

The eight animals were identified as havin
luteal cyst. They were treated with Prostaglandin
F2α analogue Dalmazin (Dextrorotatory
cloprostenol) Fatro Pharmaceutical 2ml (i/m). There
was no difference in the plasma progesterone
concentration during the study period (P>0.05). The
treatment with Dalmazin (PGF2α) changed the
plasma concentration of progesterone and it was
1.25±0.47 ng/ml, 3.88±0.88 ng/ml and 6.03±1.91
ng/ml during 1st, 2nd and 3rd week respectively (Fig.,
3). The progesterone concentration was significantly
different during different week (P < 0.5) after treatment.

Eight animals were treated with Dalmazin (Dextrorotatory cloprostenol). All animals 8(100%) were responded. The 6(75%) animals became pregnant. The remaining 2(25%) were exhibited in regular cycling process.

Table I represent the effect of Dalmazin (Dextrorotatory cloprostenol) treatment with according to age (years), body weight (kg) relationship. The three animals at the younger age (three year) with body weight of 193.3 kg showed response after 17.6 days of treatment. The animals at age of four year, body weight 160 kg showed response 32 days past treatment. The animals of six years old with a body weight of 270 kg showed response after 32 days of treatment. The 11 years old cow with a body weight of 300kg represented response after 37 days post treatment. The animals at the age of 12 years with a body weight of 280 kg give response after three days and the cows at the of 14 years with body weight of 315 kg represents the response after 10 days.

Table II & Fig., 4 explained the average birth body weight, puberty weight, and milk yield and lactation length. The mean birth body weight and puberty weight was 22.59±0.71 and 2.78±0.65 kg respectively.

The milk yields at different stages of lactation have been presented in Table II & Fig., 5. The over all lactation period in these animals was 208.95±3.74, 256.36±9.44 and 290.53±9.45 days during 1st, 2nd and 3rd lactation respectively. The mean production of the milk was 1614.12± 58.74, 2312.06± 66.87 and 2656.53±94.99 liters during 1st, 2nd and 3rd lactation respectively.

The milk yields increased with the increase in lactation number. It represents that 1st lactation has a low milk production than the 2nd and 3rd lactation number.

**Fig., 1:** The overall prevalence of cystic ovarian disease in the crossbreed cows at Multan Dairy Farm Vehari Road Multan.

**Fig., 2:** The Plasma Progesterone concentration before treatment at Multan Dairy Farm.

**Fig., 3:** The Plasma Progesterone concentration after treatment with Dalmazin (PGF2α) at Multan Dairy Farm.

**Fig., 4:** Average Birth Body Weight & Puberty Weight in Cross Breed Cows.

**Fig., 5:** The comparison of the milk production and lactation period in the cross breed cows at Multan Dairy Farm Vehari Road Multan.
Table No. I: The effect of Dalmazin treatment in the crossbreed cows suffering from luteal cyst of different body weight and age at Multan Dairy Farm Vehari Road Multan.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Breed</th>
<th>Age (Year)</th>
<th>Weight (kg)</th>
<th>Date of last Calving</th>
<th>Day after which animal responded</th>
<th>Pregnancy result</th>
<th>No. of Calving</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>¾</td>
<td>3</td>
<td>170</td>
<td>Y S</td>
<td>11</td>
<td>After 1 Service</td>
<td>Y S</td>
</tr>
<tr>
<td>2</td>
<td>5/8</td>
<td>3</td>
<td>200</td>
<td>Y S</td>
<td>08</td>
<td>After II</td>
<td>Y S</td>
</tr>
<tr>
<td>3</td>
<td>½</td>
<td>3</td>
<td>210</td>
<td>Y S</td>
<td>34</td>
<td>After II</td>
<td>Y S</td>
</tr>
<tr>
<td>4</td>
<td>¾</td>
<td>4</td>
<td>160</td>
<td>Y S</td>
<td>32</td>
<td>After I</td>
<td>Y S</td>
</tr>
<tr>
<td>5</td>
<td>5/8</td>
<td>6</td>
<td>270</td>
<td>10/1/03</td>
<td>32</td>
<td>After I</td>
<td>02</td>
</tr>
<tr>
<td>6</td>
<td>5/8</td>
<td>11</td>
<td>300</td>
<td>23/1/03</td>
<td>37</td>
<td>After II</td>
<td>06</td>
</tr>
<tr>
<td>7</td>
<td>½</td>
<td>12</td>
<td>280</td>
<td>3/1/03</td>
<td>03</td>
<td>After I</td>
<td>05</td>
</tr>
<tr>
<td>8</td>
<td>5/8</td>
<td>14</td>
<td>315</td>
<td>1/3/02</td>
<td>10</td>
<td>After I</td>
<td>08</td>
</tr>
</tbody>
</table>

Table No. II: The comparison of the milk production and lactation period in the crossbreed cows at Multan Dairy farm Vehari Road Multan.

<table>
<thead>
<tr>
<th>Birth Body Weight Mean ±SEM</th>
<th>Puberty Weight Mean ±SEM</th>
<th>Milk Yield (Litters) Mean ±SEM</th>
<th>Lactation Length (Days) Mean ±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.59±0.71</td>
<td>278.65±1.69</td>
<td>1614.12±58.74</td>
<td>208.95±3.74</td>
</tr>
<tr>
<td>2312.06±66.87</td>
<td>256.36±9.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2656.53±94.99</td>
<td>290.53±9.45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

The important feature of present study was to demonstrate the luteal cysts and the effect of Dalmazin (Dextrorotatory cloprostenol) in cross breed cows. The current study illustrate that the generally prevalence of cystic ovarian disease was 20.12% in this cross breed animals. Barlett et al. (1986) has reported that ovarian cysts in dairy cows vary form 10 - 13% and herds may have a greater incidence (30 to 40%) for brief periods (Archibald & Thatcher, 1992).

Only 8 cases out of 18 cases (44.44%) were correctly diagnosed through rectal palpation of clinical forms of luteal cyst by progesterone analysis and this is supported by previous studies where misdiagnoses of ovarian function ranged from 20 to 50% (Grunert, 1993).

Grunert (1993) has described rectal palpation as the main method for clinical evaluation of ovarian activity in dairy herds also described that it may cause high proportion of misdiagnosis in incorrectly treated animals. But according to Pieterse et al. (1990), ultrasound scanning can be used to avoid misdiagnosis and progesterone determination in milk or blood. Even for an experienced practitioner, rectal palpation can create difficulties to differentiate between follicular and luteal cyst (Farin et al., 1992). However, with identification of greater than 90% of luteal and about 75% of follicular cysts, use of transrectal ultrasonography can generate accurate diagnosis (Farin et al., 1992).

Follicular and luteal cysts also can be classified based on serum progesterone profile (Farin et al., 1992). The mean (se) plasma progesterone concentration was lower in the cows with follicular cysts than in those with luteal cysts (0.29 [0.05] v 3.90 [0.63] ng/ml; P<0.05) (Douthwaite & Dobson, 2000). Administration of PGF2α can induce the regression of luteal cysts (Nanda et al., 1998). In present study the occurrence of the luteal cyst was found to be 8 (44.44%) of the 18 cases were correctly diagnosed by plasma progesterone profile in the blood. Booth (1988) indicated the progesterone assay that the diagnosis was correct in 54 per cent of the luteal cases in diary cow. The reported data indicates that the percentage of luteal cyst is greater than our study cross breed cows. The thick walled, fluid filled
structure and ≥25 mm in diameter luteal cysts secrete progesterone in normal or above normal amounts. Mostly these luteal cysts are formed through luteinization of follicular cysts (Garverick, 1997) which cause infertility when it maintains systemic progesterone at concentrations that inhibit that LH surge and ovulation. In heifers, during the estrous cycle and early pregnancy, 79% of otherwise normal CL contains cavities ranging from less than 2 to greater than 10 mm in diameter observed through ultrasonographic examination. Luteal cysts had thicker walls (5.3 [0.04] v 2.5 [0.2] mm; P<0.0001), and the wall thickness of all the cysts was positively correlated with plasma progesterone concentration (r=0.52, P<0.0004). Cows with luteal cysts had more additional follicles greater than 5 mm in diameter (P<0.01). In cows with follicular cysts and other follicles greater than 5 mm in diameter, the mean oestradiol concentration was 7.9 (1.8) pg/ml compared with 24.2 (3.1) pg/ml (P=0.002) in cows without other follicles greater than 5 mm in diameter on either ovary (Douthwaite & Dobson, 2000).

The current study showed that the prostaglandins were luteolytic agent in the cross breed cows as confirmed by Lauderdale (1972).

An effective treatment for ovarian cysts is the use of injections of both GnRH and PGF2α called Ovsynch; a protocol for synchronizing ovulation in lactating dairy cows (Pursley et al., 1997).

To synchronize estrus manifestations and improve the use of biotechnologies, prostaglandins are widely used. These prostaglandin causes corpora luteal to regress during the restriction phase of the estrous cycle, where the CL is responsive to consequent decrease in the levels of progesterone and estrous manifestations occurrence followed by ovulation often 2 to 5 days of the administration (Nebel & Jobst, 1998).

In the present study the pregnancy rate was 75 percent in the luteal cyst disorder animals after treatment. The percentage of the regular cycling was 25%. While the early reports show 50 per cent pregnancy rate (Douthwaite & Dobson, 2000). It was cleared that the pregnancy rate in the present study was greater than reported in the literature. The percentage of the responded animal was hundred percent in the current study, while Booth (1988) reported that 74 per cent of the confirmed cystic cases responded to treatment within two weeks. This difference may be due to the follow up periods as this was 37 day in the present study.

The data on the lactation milk yield was 1614.12 ± 58.74, 2312.06 ± 66.87 and 2656.53±94.99 litters during 1st, 2nd and 3rd lactation number respectively. The overall average lactation period in these animals was 208.95±3.74, 256.36±9.44 and 290.53±9.45 days during 1st, 2nd and 3rd lactation number respectively. It was observed that with the increase of milk yield level the lactation period was also increased.

In conclusion the incidence of postpartum disorders studies by rectal palpation were 15 (4.64%), follicular cyst, 11 (3.40%), corpus luteum 12 (3.71%), persistent corpus luteum 12 (3.71%), Anoestrous 13 (4.02%), and cystic overy 2 (0.61%) in the cross breed cows. The results of the rectal palpation were confirmed by plasma progesterone concentration. The concentration of the progesterone before treatment in the blood was 1.44±0.44 ng/ml, 1.12±0.56 ng/ml and 0.56±0.23 ng/ml during 1st, 2nd and 3rd week respectively. The 8 cows were found as having luteal cyst. These were treated with Prostaglandin F2α analogue Dalmazin (Dextrorotatory cloprostenol). The treatment with Dalmazin (PGF2α) changed the plasma concentration of progesterone and it was 1.25±0.47 ng/ml, 3.88±0.88 ng/ml and 6.03±1.91 ng/ml during 1st, 2nd and 3rd week respectively. All the treated animals were responded, 75% were pregnant and 25% were in regular cycling. The dalmazin (PGF2α) treatment enhanced the reproductive efficiency of the crossbreed cows, which were suffering from luteal cyst.

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