REPEATED SUPEROVULATION IN CATTLE AT SHORT INTERVALS

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ABSTRACT
Repeated superovulation at short intervals was done in 7 Rathi and its crossbred heifers. CIDR-B was implanted in the vagina of heifers and they were superovulated with 8 divided 12 hr IM decreasing doses of porcine FSH (Super-Ov, Ausa International USA) starting from day 8 of the implant. At 6th injection of FSH, 5ml of prostaglandin was injected IM to induce estrus. Embryos were recovered non-surgically on day 7 of insemination during a superovulatory estrus. Fourteen days after embryo recovery the CIDR-B was placed again and the same treatment was repeated thrice. The mean ovulatory response, total embryo recovery and transferable grade embryo decreased at the fourth treatment but the differences were non-significant indicating that superovulation can be done in cattle at short intervals.

INTRODUCTION
Repeated superovulation is needed for outstanding donors for their optimum use in a MOET programme. It is generally considered that there should be a minimum 60 days interval between two repeated treatments of the same donor (Totey et al., 1992; Misra et al., 1997) and that the superovulation response, fertilization rate and embryo recoveries decline after 1-3 such treatments (Hasler et al., 1983). Contrary to these reports other workers suggested that neither parameters including embryo recoveries decline after repeated superovulation (Lamberson and Lambeth, 1986; Jordt and Lorenzini, 1988). The time interval between two superovulation treatments has been a subject of limited study and Sacher et al. (1987) were of the view that the embryo recoveries decline after repeated treatment if the inter treatment interval was reduced to 40-50 days. More recent reports (Sekizawa et al., 1992; Oikawa et al., 1998) suggest that superovulation protocols may be undertaken repeatedly and with an inter treatment interval of as less as 33 days without affecting the embryo recoveries. The present study was aimed at evaluating repeated superovulation in Rathi cattle at short intervals using a progestagen implant: CIDR-B and porcine FSH.

MATERIAL AND METHODS
This study was conducted on 7 Rathi heifers (aged 3-6 years) of the department of Veterinary Obstetrics and Gynaecology, Bikaner. The animals were maintained under standard conditions in sheds. After observing 2 estruses the animals were repeatedly superovulated at short intervals for four times using the method described by Oikawa et al. (1998). Briefly, each animal was implanted with a progestagen implant (CIDR-B, Inter AG Hamilton, NJ) irrespective of the stage of oestrous cycle and superovulated by injecting eight 12 hourly IM decreasing doses (2.0, 2.0, 1.5, 1.5, 1.0, 1.0, 0.5 and 0.5 ml) of a porcine FSH(Super-Ov, Ausa International, USA) on day 8, 9, 10 and 11 of the placement of the implant. Animals were induced to estrus by injecting an IM dose of 5 ml of a prostaglandin (Iliren, Hoechst Roessle Vet, Germany) at the time of 6th injection of FSH. The implant was withdrawn at the same time. Animals were inseminated twice with frozen semen at 12 hourly intervals intervals starting from 8-10 hours after visual estrus detection. The ovulatory response was detected by counting the number of C.I on the ovaries one day before the non-surgical recovery of embryos (Purohit et al., 2000) on day 7 of estrus. Embryos were graded as per: Stringefellow and
Seidel (1990) and considered as transferable grade embryos (TGE) when they were in morula or blastocyst stage, were symmetrical, had even granulation, had no deformation in zona pellucida and no blastomere extrusion.

Immediately after non-surgical embryo recovery donors were injected with a dose of PG (5ml, Iliren IM). Irrespective of estrus, animals were implanted with the same progestagen implant on day 14. The same superovulatory treatment was repeated for 3 occasions and the embryo recoveries recorded for all the four superovulatory treatments done at short intervals.

The mean ovulation response and the total and transferable grade embryos were compared between the I to IV repeated treatments by standard statistical procedures described previously (Snedecor and Cochran, 1975).

RESULTS AND DISCUSSION

All animals showed a superovulatory response (CL more than 3) during all the repeated treatments (28) except animal number 4 which had only 1, 3 and zero CL during the first three treatments. The estrus induction interval varied between 30.45±0.75 to 37.0±4.50 hours and estrus duration 22.0±2.40 to 30.55±3.65 hours for the superovulatory estrus. The mean ovulatory response, total and transferable grade embryos recovered during the four repeated treatments (Table 1) showed that the decline in these parameters was non-significantly different. Comparison of these parameters ('t' test) also showed that the differences were non-significant (P>0.05).

However, the number of unfertilized ova were significantly higher (P<0.05) during the fourth repeated treatment. During the first two superovulatory treatments the proportion of animals showing a high ovulatory response (number of CL >15) was 14.29% (1/7) whereas during the third and fourth treatments the respective proportion of animals showing a high response was nil. During the fourth repeated treatment however, 14.29% (1/7) animals showed a mild (number of CL <5) ovulatory response. One month after the last repeated treatment one animal developed polycystic ovary and was resolved by treatment with GnRH.

The present findings in terms of ovulatory responses, total embryo/ova recovery and transferable grade embryo recovery are similar to previous findings of Sekizawa et al. (1992); Oikawa et al. (1998) and Chandra et al. (2002) in their repeated superovulation at short intervals. Lamberson and Lambeth (1986); Jordt and Lorenzini (1988) and Vyas (1998) had also recorded similar results although they had attempted repeated superovulation at long intervals. It was concluded that repeated superovulation can be performed in cattle at short intervals with little decrease in embryo recovery.

Table 1. Mean ovulatory response, total embryos/ova recovered and transferable grade embryos recovered during four repeated superovulation treatments at short intervals in Rathi heifers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ovulatory response (number of CL)</th>
<th>Total embryo recovery (TE)</th>
<th>Transferable grade embryo recovery (TGE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10.6±1.67</td>
<td>8.57±1.70</td>
<td>7.28±1.32</td>
</tr>
<tr>
<td>II</td>
<td>9.71±1.26</td>
<td>7.85±1.20</td>
<td>6.14±0.89</td>
</tr>
<tr>
<td>III</td>
<td>9.85±1.78</td>
<td>7.57±1.67</td>
<td>6.57±1.49</td>
</tr>
<tr>
<td>IV</td>
<td>8.0±1.0</td>
<td>6.14±0.88</td>
<td>4.28±0.42</td>
</tr>
<tr>
<td>Overall</td>
<td>9.39±1.28</td>
<td>7.82±1.33</td>
<td>6.07±0.57</td>
</tr>
</tbody>
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REFERENCES