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Evaluation of ovarian response and embryo production pattern in Ongole cows

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ABSTRACT

The present study was undertaken to evaluate the relative distribution of ovarian response and embryo recovery in a large scale embryo transfer programme in Ongole (*Bos indicus*) cows. Parous Ongole cows (58) were subjected to super ovulatory treatment by administering 200 mg NIH-FSH-p1 in a twice daily descending dose schedule for 4 days and induction of luteolysis at 48 and 60 h after initiating treatment. Non-surgical embryo collection was performed on day 7 after superestrus; 94.83% cows responded to superovulation with a mean of 13.53±0.79 ovulations. The mean total ova/embryo recovery was 10.09±0.93 of which 72.79% were transferable. Grade 1 embryos (73.02%) and compact morula (72.78%) were the predominant category in this study.

Key words: *Bos indicus*, Compact morula, Embryo, Superovulation

Successful integration of embryo transfer technology into a breeding programme largely depends on the number of transferable embryos recovered as well as the percentage of embryos suitable for freezing. However, uncertainty as well as variability in superovulatory response within and between donors continued to be a frustrating task for extensive exploitation of superior females to realize more number of embryos (Mapletoft *et al*. 2002). It has been reported that while majority of cows produce fewer than four viable embryos, only about 15% of donors yield more than 15 embryos (Silva *et al*. 2009). This unpredictability in ovarian response and embryo production pose severe logistic problems and contribute to high cost of embryo production. The response to superovulation is mainly dependant on the status of ovarian follicles at the commencement of gonadotrophin treatment (Mapletoft *et al.* 2002). Perusal of the literature indicated that the developmental rate of embryos in superovulated cattle vary considerably presumably due to considerable time gap between the first and last ovulation (Purwantara *et al*. 1994). The present study is an attempt to evaluate the relative distribution of ovarian response, embryo recovery and the developmental pattern of embryos recovered in a large scale superovulation programme in Ongole (*Bos indicus*) cattle.

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MATERIALS AND METHODS

Parous, clinically normal, cyclic, 6–14 years old Ongole cows (58), were used as donors. Based on the milk yield the daily ration of each animal consisted of 3–5 kg high protein feed, 20–30 kg chopped fodder (*Sorghum bicolor* or *Pennisetum purpureum*) and paddy straw *ad lib*. They were also sent for grazing daily between 0900 and 1500 h to the adjoining pasture lands and water was available free choice. Calves were allowed to suckle the dams and hand milking was practiced twice a day.

The donors were subjected to superovulation treatment on day 10 of their natural cycle with or without steroid pre-treatment or follicle ablation. The treatment involved administration of 200 mg NIH – FSH – P1 (folltropin-V) i/m in a twice daily descending dose schedule (40, 40, 30, 30, 20, 20, 10 and 10 mg) at 0600 and 1800 h for 4 days. Luteolysis was induced by administering 50 mg dinoprost tromethamine (lutalyse) at 48 h (PGF1) and 60 h (PGF2) after initiating gonadotrophin treatment. The donors were inseminated at 48 and 60 h post-PGF1 administration with frozen thawed semen in a 0.5 ml straw with at least 15 million live sperms post-thawing. Cows exhibiting prolonged standing estrus received an additional third insemination 12 h after the second one. Non-surgical embryo collection was performed 7 days after the onset of standing estrus (super estrus). Prior to embryo collection the number of corpora lutea and anoulatory follicles >10 mm (AFL) on each ovary were estimated per rectum. Embryo collection from donor cows was carried out with minor modifications as per Hasler *et al*. (1983). The embryos were isolated, evaluated and classified based on the stage of development as unfertilized ova, early morula, compact morula, early blastocyst, blastocyst, expanded blastocyst, hatched blastocyst, empty zona and degenerated embryo (Lindner and Wright 1983). Based on the morphological features, the viable (transferable) embryos were further classified as grade 1, 2 and 3 embryos (Mapletoft 1986). The data collected was analyzed as per Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

The pattern of multiple ovulations, embryo production and developmental stages of embryos is presented in Table 1. Only 5.17% cows failed to respond to superovulation. This is much lower than the 43% non responders reported earlier in cattle (Ranjna *et al*. 2004). Cumulatively, 8.62% cows had ≤5 ovulations (poor responders), 34.48% had 6–10 ovulations (average responders), 27.59% had 11-15 CL (good responders) and 29.3% had >15 CL (excellent responders). These results are in agreement with an earlier report of 53–85% animals producing ≤7 ovulations following superovulation (Kohram *et al*. 1998). In general there was a pronounced variation in superovulation response (range, 2–27 CL) and much of this variability can be considered either intrinsic or extrinsic to the donor (Kafi and McGowan 1997). In cattle various intrinsic factors that influence superovulation response are: genetic (Snyder 1986), presence or absence of dominant follicle (Guilbault *et al*. 1991), circulating levels of estrogen (Saumande *et al*. 1978), progesterone (Dhanani *et al*. 1991) etc. Other factors, considered as extrinsic to the donor animal that might influence ovarian response include the characteristics of gonadotrophin preparations, duration of treatment, timing of treatment with respect to estrous cycle and the total dose of gonadotrophin used (Mapletoft *et al*. 2002).

Table 1. Ovarian response and embryo production patterns in Ongole cows

Attributes	Treatment with FSH
No. of superovulation treatments	58
No. of responded treatments (%)	55 (94.83)
Number of ovulations (CL)	13.53±0.93
% Poor responders (≤ 5 CL)	8.62
% Average responders (6–10 CL)	34.48
% Good responders (11-15 CL)	27.59
% Excellent responders (> 15 CL)	29.30
Total ova/embryos (%)	10.09±0.93 (74.59)
No. of cows yielding	
No embryos (%)	01 (0.55)
1–5 embryos (%) 6–10 embryos	13 (7.15)
> 10 embryos (%)	22 (40.00)
Viable embryos (%)	19 (34.54)
No. of cows yielding	7.35±0.77 (72.79)
No viable embryos (%)	3 (5.56)
1–5 viable embryos (%)	23 (42.59)
6–10 viable embryos (%)	15 (25.42)
> 10 viable embryos (%)	13 (24.07)
Fertilization rate	94.77%
Grade 1 embryos (%)	5.36±0.64 (73.02)
Grade 2 embryos (%)	1.49±0.23 (20.30)
Grade 3 embryos (%)	0.49±0.08 (6.68)
Early morula (%)	5.45
Compact morula (%)	72.78
Early blastocyst (%)	9.15
Blastocyst (%)	6.45
Expanded blastocyst (%)	6.68

Of the 55 animals subjected to non-surgical embryo collection only 1 animal (6.67%) did not yield embryos. This is much lower than the 35.0% (Kanuya *et al*. 1997) nil embryo recovery observed following flushing. Cumulatively, 25.45% cows yielded less than 5 embryos, 40.00% cows yielded 6–10 embryos and 34.54% cows yielded >10 embryos. In general there was wide variation in total ova/embryo recovery (range, 0–27) in the present study. This is in agreement with our earlier study in Ongole (*Bos indicus*) cows that the total ova/embryo recovery was as high as 80–100% in 32% and as low as 20% in 25% of the donors flushed (Kasiraj *et al*. 2000). Looney (1986) also reported that 30% collections yielded nearly 70% of the embryos. Thus, the wide variation in embryo recovery may in turn be related mainly to the individual variation in response to superovulation treatment.

A high fertilization rate of 94.77% observed in this study indicated the fertility rate of bulls as well as the absence of any deleterious effect of superovulation protocol on sperm transport. In keeping with our results, Baracaldo *et al*. (2000) reported 88–90% fertilization rate with different treatment regimen in superovulated cows.

Classification of viable embryos recovered in the present experiment revealed that compact morula and blastocyst comprised the highest number (87.87%) and the remaining were expanded blastocyst (6.68%) and early morula (5.45%).We are in agreement with Lindner and Wright (1983) who reported 3% morula, 90% compact morula and blastocyst and 6% expanded blastocyst on day 7 after super estrus. Another study in buffaloes flushed on day 5.5–6.0 after the onset of estrus showed a recovery of 48% morula, 37.5% blastocyst and 10.8% hatched blastocyst (Misra *et al*. 1990). Thus the study showed that the treatment protocol had no deleterious effect on the developmental rate of embryos.

Categorisation of embryos into different grades based on various morphological criteria revealed that grade 1 embryos comprised the highest number (73.02%), followed by grade 2 (20.30%) and grade 3 (6.68%). These results are in agreement with earlier reports that out of 6.2 viable embryos recovered 5.9 (95.16%) were grade 1 and grade 2 and the remaining 0.4 (4.84%) were grade 3 embryos (Baruselli *et al*. 2006). In contrast, Kohram *et al*. (1998) reported only 2.6 grade 1 and 1.7 grade 2 embryos. The variation in the number of different grades of embryos may be related to differences in various response parameters like ovulation rate and total ova/embryo recovery with different superovulation protocols used.

It may be concluded that the native Ongole (*Bos indicus*) are equally efficient as exotic cows in superovulation response and the hormonal protocol followed in this study has no deleterious effect on the rate of development and quality of embryos recovered.

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