ULTRASONIC MONITORING OF FOLLICLES AND CORPORA LUTEA DURING SYNCHRONIZATION IN SUMMER ANOESTROUS NILI RAVI BUFFALOES AND THEIR SUBSEQUENT SUPEROVULATORY RESPONSE

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ABSTRACT

In the first experiment, effect of GnRH and $PGF_{2\alpha}$ given intramuscularly, 9 days apart, was observed on induction of ovulation and synchronization of oestrus in anoestrous buffaloes during summer (n=2). Ovarian follicles and corpora lutea were monitored on every other day basis, using B-mode real time, transrectal ultrasonography. Oestrus detection was carried out twice daily. The diameter of the largest follicle on the day of administration of GnRH averaged 9 ± 0 mm. These follicles ovulated within 48 h after injection of GnRH. Interval to oestrus after injection of $PGF_{2\alpha}$ was 63 ± 11 h. In the second experiment, effect of follicle stimulating hormone (FSH-p) on follicular development in buffaloes during summer (n=2) was observed. FSH-p (total of 40 mg) was administered intramuscularly in divided doses, twice daily on Days 10, 11, 12 and 13. $PGF_{2\alpha}$ (2 ml) was injected on Day 13. Ovarian follicles and corpora lutea were monitored on daily basis using real time, transrectal ultrasonography. Oestrus detection was carried out twice daily. Superovulatory response was measured and analyzed by comparing follicular development on Day 10, i. e. beginning of FSH-p treatment, (before) and on Day of next oestrus (after). Superovulatory response was confirmed by determining number of corpora lutea on Day 7 after oestrus. Interval to oestrus after injection of $PGF_{2\alpha}$ was 37 ± 10 h. Mean number of small follicles decreased ($P<0.05$) 'after' FSH-p treatment than 'before'. Mean number of medium and large follicles and corpora lutea increased ($P<0.05$) 'after' FSH-p treatment than 'before'. It is concluded that protocol of GnRH-$PGF_{2\alpha}$ can induce ovulation and oestrus in buffaloes and reasonable superovulatory response to FSH-p can be achieved during summer when given during mid luteal phase.

Key words: Synchronization of oestrus, superovulation, ultrasonography, anoestrous buffaloes.

INTRODUCTION

Protocols for synchronization of oestrus have been reviewed in cattle (Twagiramungu *et al*., 1995) and in buffaloes (Baruselli *et al*., 2003). Similarly, protocols for the superovulation using either PMSG or FSH have been established in cattle (Lindsell *et al*., 1986; Goulding *et al*., 1990) and in buffaloes (Karaivanov, 1986). Superovulatory response in the buffalo was quite variable and differed with ovarian status, season, age and the stage of the cycle at which administration of exogenous hormones was initiated (Rahil *et al*., 1989; Manik *et al*., 1994; Taneja *et al*., 1995b). When initiated in mid luteal phase, FSH treatment resulted in better response than when initiated it in early luteal phase in cattle (Goulding *et al*., 1990). More recently, synchronization protocols with fixed time inseminations in buffaloes have been shown to yield good results (Baruselli *et al*., 2003). Use of ultrasonic imaging of ovarian follicular and luteal development has greatly enhanced our understanding of reproductive physiology in cattle (Pierson and Ginther, 1987) and buffaloes (Baruselli *et al*., 1997). Development of synchronization protocols for anoestrous buffaloes, using GnRH and $PGF_{2\alpha}$ and superovulatory response against FSH-p can be of practical significance as these hormones can control ovarian follicular and luteal functions.

The first objective of the present study was to determine the response of GnRH and $PGF_{2\alpha}$ for induction of ovulation and synehronization of oestrus in anoestrous Nili Ravi buffaloes measured in terms of follicular and luteal development by using real time, transrectal ultrasonography. The second objective of the study was to characterize the superovulatory response of FSH-p when given during mid luteal phase in Nili Ravi buffaloes.

MATERIALS AND METHODS

Two anoestrous Nili Ravi buffaloes, maintained under uniform conditions of feeding and management

at the experimental herd of University of Veterinary and Animal Sciences, Lahore were used during summer (May to June) of 2004. Buffaloes were in moderate body condition and at the end of their lactation period. During both the experiments oestrus detection was carried out twice daily by using a teaser bull.

In the first experiment, each buffalo (n=2) received 2 ml of GnRH (Dalmeraline, FATRO, Italy) given intramuscularly and 2 ml of $PGF_{2\alpha}$ (Delmazine, FATRO, Italy) given intramuscularly 9 days after injection of GnRH (Fig. 1). In the second experiment, each buffalo (n=2) received FSH-p (Sigma; total of 40 mg), given intramuscularly, in divided doses, twice daily on Days 10, 11, 12 and 13 of synchronized oestrus cycle. Injection of $PGF_{2\alpha}$ (Delmazine, FATRO, Italy) 2 ml, was given intramuscularly on Day 13 (Fig. 1). Transrectal ultrasonography with a B-mode scanner (Falco Vet 100; Pie Medical; Holland) fitted with 8 MHz linear-array transducer was used for monitoring ovarian follicles and corpora lutea on every other day in the first experiment and daily in the second experiment. Single person scanned reproductive tract and took the measurements of ovarian structures by built-in calipers. Ovulation was considered as sudden disappearance of large sized follicle from the ovary.

Ovarian follicular response was analyzed by comparing each sized follicles, i.e. small (4– 6 mm), medium (6 – 9 mm) and large (10 mm and above) on Day 10 (before) and on the Day following oestrus (after). Superovulatory response was confirmed by determining number of corpora lutea on Day 7 after oestrus using B-mode real time, transrectal ultrasonography. Mean values (± SEM) for various parameters were computed. Paired t test was applied for statistical analysis.

RESULTS

In the first experiment, GnRH-$PGF_{2\alpha}$ protocol induced ovulation and oestrus in anoestrous buffaloes during summer. The diameter of largest follicles on day of administration of GnRH averaged 9 ± 0 mm. These follicles ovulated within 48 h after injection of GnRH and subsequently formed CL. Interval to oestrus after injection of $PGF_{2\alpha}$ averaged 63 ± 11 h.

In the second experiment, interval to oestrus after injection of $PGF_{2\alpha}$ was 37 ± 1.1 h. Comparison of follicles and corpora lutea on Day10 (before FSH-p) and day of following oestrus (after FSH-p) is given in Fig. 2. The mean number of small follicles decreased ($P<0.05$) from 2.0 ± 1 before FSH-p treatment to 0.75 ± 0.5 after FSH-p treatment whereas, the mean number of medium and large follicles and corpora lutea increased ($P<0.05$) from 1.00 ± 0.4, 0.25 ± 0.2 and 1.0 ± 0 before FSH-p treatment to 2.2 ± 1.0, 2.7 ± 0.6 and 5.5 ± 1.7 after FSH-p treatment, respectively.

DISCUSSION

In Pakistan, perhaps this is the first ever study in Nili Ravi buffaloes in which follicular and luteal development has been monitored by using B-mode real time, transrectal ultrasonography. First experiment of the present study was designed to determine if GnRH and $PGF_{2\alpha}$ treatment in anoestrous buffaloes, given nine days apart during summer, induces ovulation and resumes cyclicity. Indeed, this protocol worked well, as both the buffaloes ovulated their largest follicles and came in oestrus (response was 100%), although only two animals were used. This method synchronized the oestrous cycle of about 70-80% of the cyclic cows (Twagiramungu *et al*., 1995) and appears to be effective

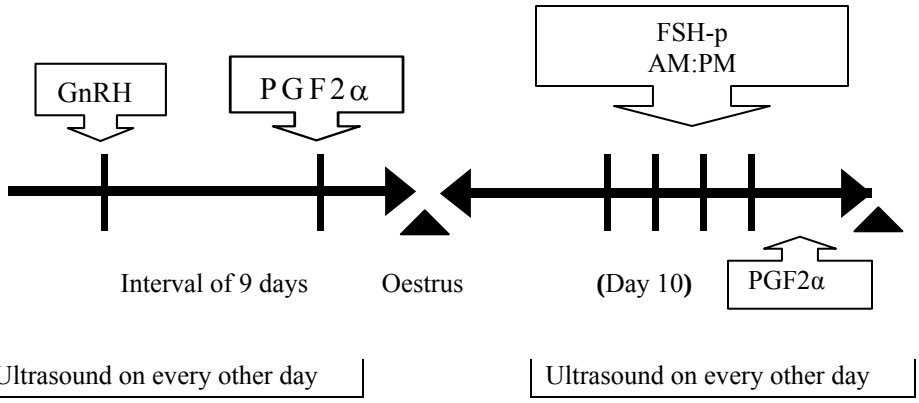

Fig. 1: Protocol of 1st and 2nd experiment in anoestrous Nili Ravi buffaloes during summer.

for controlling ovarian follicular and luteal functions. GnRH and its agonist act on ovarian follicular development and CL formation indirectly via the induced release of pituitary LH and FSH (Conn and Crowley Jr, 1991). Administration of GnRH causes the large follicles to ovulate and induces emergence of a new follicular wave within 3 to 4 days after treatment at any stage of the oestrous cycle in cattle. The large follicle of this wave becomes ovulatory follicle after $PGF_{2\alpha}$ induced luteolysis. The reason we chose nine days interval between GnRH and $PGF_{2\alpha}$ as compared to usual interval of seven days, reported in cattle (Goulding *et al*., 1990; Pursley *et al*., 1995), was that the CL in buffaloes may be more developed by nine days as its sensitivity to luteolytic effects of $PGF_{2\alpha}$ increases with time (Brito *et al*., 2002).

The ovulation in response to GnRH treatment in postpartum buffaloes was 60% (Baruselli *et al*., 2003) and in cattle it was 85% (Wiltbank *et al*., 1997). The interval after GnRH to ovulation was 48 h in the present study, while 30-32 h has been reported in cattle (Pursley *et al*., 1995) and 36 h in another study in buffaloes (Baruselli *et al*., 2003). Therefore, it appears that GnRH-$PGF_{2\alpha}$ protocol can synchronize anoestrous buffaloes; however, regular heat detection is necessary. Recently, another protocol (GnRH-$PGF_{2\alpha}$-GnRH i.e. ovulation synchronization) has been reported in cattle (Wiltbank *et al*., 1995) and buffaloes (Baruselli *et al*., 2003) as well, where difficulty of heat detection has been overcome and fixed time insemination is preferred.

The second experiment was designed to assess the superovulatory response of FSH-p, when given on Day 10 of oestrous cycle, in the buffaloes of the first experiment in which cyclicity was induced by using GnRH-$PGF_{2\alpha}$ protocol. The superovulatory response in these buffaloes was evaluated by number of corpora lutea formed on Day 7 after the oestrus, using real time, transrectal ultrasonography. In our study, 5-6 corpora lutea were comparable with those reported earlier in buffaloes (Rahil *et al*., 1989; Karaivanov *et al*., 1990) but lower than 12 corpora lutea documented in cattle (Goulding *et al*., 1990). The optimal superovulatory response recorded in the present study could be due to prevention of atresia and enhanced selection of follicles. This is evidenced by the fact that number of small follicles decreased, whereas the medium and large sized follicles increased, following FSH-p treatment.

On the other hand, earlier studies in buffaloes (Taneja *et al*., 1995a) and in cattle (Guilbault *et al*., 1991; Huhtinen *et al*., 1992) reported decreased superovulatory response when FSH treatment was initiated in the presence of dominant follicle which is suggested to have inhibitory effect on the other follicles of the wave. The differences in the superovulatory response may be due to the individual variability of the animal or species. In the first and the second experiment, intervals of oestrus after administration of $PGF_{2\alpha}$ were 63 ± 11 and 37 ± 1.1 h, respectively. This difference can be related to the size of corpus luteum and follicular status before the administration of $PGF_{2\alpha}$ (Brito *et al*., 2002).

Taken together, it is concluded that protocol of GnRH-$PGF_{2\alpha}$ can induce oestrus and ovulation in buffaloes and reasonable superovulatory response to FSH-p can be achieved during summer when given during mid luteal phase. However, the number of animals used in the present study was too small to draw any conclusion. It is suggested that GnRH-$PGF_{2\alpha}$ protocol be repeated, using larger number of buffaloes alongwith their fertility.

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