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SUPEROVULATORY RESPONSE IN SUMMER ANESTRUS BUFFALOES AND CATTLE TREATED WITH ESTRUS SYNCHRONIZATION PROTOCOL

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ABSTRACT

Summer anestrus is a major problem of dairy animals in tropical and subtropical countries. In such animals superovulation for embryo transfer is unsuccessful. This study was done to evaluate the superovulatory response in dairy animals during summer season after estrus resumption. In first stage of the study, four non-cyclic animals (two buffalo, two cattle) were given Progesterone (P4) exposure through Control Internal Drug Releasing (CIDR) device and were monitored for estrus resumption through ultrasonography. Out of four, three animals showed estrus signs. It was observed, at time of CIDR removal, the animals having 6mm or bigger follicles on ovaries expressed heat signs properly. In second stage, effect of FSH on follicular development was observed. In estrus resumed animals, from day 10 to day 13 of estrous cycle, twice daily FSH-p (5mg Armour units) was administered intramuscularly. FSH countered successfully follicular dominance and follicular development pattern was same in cattle and buffalo. Double insemination was done at 12 hrs intervals after observing heat signs. In third stage, embryo recovery was observed, 7 days after first AI. In Cattle four embryos were recovered but in buffalo no embryo could be recovered due to cervical abnormality. Ovulations were confirmed by observing 3 CL through ultrasonography. So, CIDR protocol could be used in summer anestrus animals and these animals could be used for superovulation and embryo collection.

Keywords: Superovulation, FSH-p, CIDR, Ultrasonography, Summer anestrus.

INTRODUCTION

Pakistan is an agriculture based country and livestock contribution in its agricultural GDP is 53.2% (Anonymous., 2011). Pakistan is fifth largest milk producing country in the world but the reproductive performance of its dairy animals is usually affected by severe summer conditions. During summer, most of the animals are in non-cyclic (anestrus) condition. Different protocols have been studied to overcome the summer anestrus problem. Protocols for synchronization of oestrus have been reviewed in cattle (Patterson *et al.*, 2002) and buffalos (Baruselli *et al.*, 2003). One of the protocols is by Progesterone (P4) block on LH surge through Control Internal Drug Release (CIDR) application (Cerri *et al*., 2009; Dias, 2008). CIDR application for estrus resumption in different species has also been reported; cattle (Savio *et al.*, 1993) buffalo (Singh, 2003) and sheep (Knights *et al.,* 2001).

In Pakistan, animals with different genetic potential (ranging from 2 liters to 25 liters daily milk yield) are available (Afzal *et al*., 2007). During summer season, due to low conception rate, animals with good genetics are routinely being slaughtered. A.I technique is already being utilized to propagate good genetics from male side. In order to harvest the maximum genetic potential from both male and female side, we need to emphasize on embryo transfer technique. Due to summer anestrus problem, the effective utilization of this embryo transfer technology is questionable. During summer season, it is difficult task to select the good donor animals for super ovulation protocol. No reports for superovulatory response in cyclicity induced summer anestrus animals are available. This study is, hence, designed to evaluate the super ovulatory and embryo recovery response after estrus induction in summer anestrus animals.

MATERIALS AND METHODS

Animal Management: During summer season, four non-cyclic animals, two cattle and two buffalo, were selected from the University of Veterinary and Animal Sciences, experimental herd station. Acyclicity in these animals was confirmed by absence of luteal tissue on ovaries by ultrasound scanning. All animals were maintained under uniform conditions and were offered chopped green fodder, 10% of the live body weight.

Estrus induction: Ovarian cyclicity in the non-cyclic animals was induced by giving Progesterone (P4) exposure. The CIDR device (Eazi-Breed CIDR®, Pfizer Animal Health), containing 1.38 grams of P4 in porous membrane, were placed in the vagina. After six days, 2 ml of $PGF_{2\alpha}$ (Delmazine, FATRO, Italy; 0.075mg/ml) was administered intramuscularly and seven days after CIDR was removed (Figure 1, A). Within 48 hours after CIDR removal, the animals with clear mucus discharge along with swelling of vulva were declared as estrus

animals. Based on heat signs the estrus intensity was graded from 1 to 3.Grade 3 was used to explain animal having copious mucus discharge, pronounced vaginal swelling and rigid horns; while grade 1 describe no discharge, no vaginal swelling and flaccid horns.

Super Ovulation: The animals that showed heat signs within 36 hrs after CIDR removal were selected for superovulation protocol. Ovaries of the animals (n=2) were super-stimulated by giving exogenous source of Follicular Stimulating Hormone (FSH-p; Sigma Aldrich, F-2293). Each animal was administered a total of 40 mg Armour units FSH-p, in repeated doses for four consecutive days. The FSH-p was injected intramuscularly in morning/evening repeated doses (5mg - Armour units) on 10, 11, 12 and 13 of estrous cycle (Ayres *et al*., 2011). On day 13 of estrous cycle a $PGF_{2\alpha}$ injection (2ml, Delmazine, Fatro, Italy) was injected intramuscularly, to break P4 block by regressing luteal tissue. After heat detection double insemination (morning/evening) was done (Figure 1, B)

Embryo Recovery: Embryos were retrieved from horns of the super ovulated animals, seven days after insemination. Flushing solution Ultra-embryo (ICPbio Emcare) was used to flush the embryo (Hayakawa *et al*., 2009). The quality of embryos was evaluated under stereomicroscope and embryos with initial development but not fully develop according to the embryonic stage were categorized as B grade and embryos with more pronounced degeneration, that it may not be possible to determine the exact developmental stage, were declared as degenerated.

Ovarian Scanning: Throughout the study, ovarian status was monitored by ultrasonography. Ultrasound scanning was performed by using ultrasound machine fitted with a 5 MHz, B-mode linear-array transrectal scanner (FalcoVet 100; Pie Medical; Holland). Follicular dynamics after ovarian stimulation by FSH-p was monitored by scanning ovaries daily. Estrous cyclicity and number of ovulations were confirmed by scanning the presence of CLs on both ovaries.

RESULTS

In stage one of the experiments to induce estrus by CIDR application, out of four, two animals showed estrus signs within 36 hrs and one animal showed estrus signs after 60 hrs of CIDR removal. One animal did not show heat signs in response to CIDR-PGF2α protocol. Ovulation time after estrus expression was a little prolonged in buffalo as compared to cattle (Table 1). To evaluate the heat expression in response to hypothalamic exposure with P4, it was observed that at time of CIDR removal, the animals having medium size follicles (5-6mm) on ovaries expressed well defined heat signs. The animal with smaller size follicles (2-3mm) at time of CIDR removal did not show heat signs or heat expression was delayed and poor.

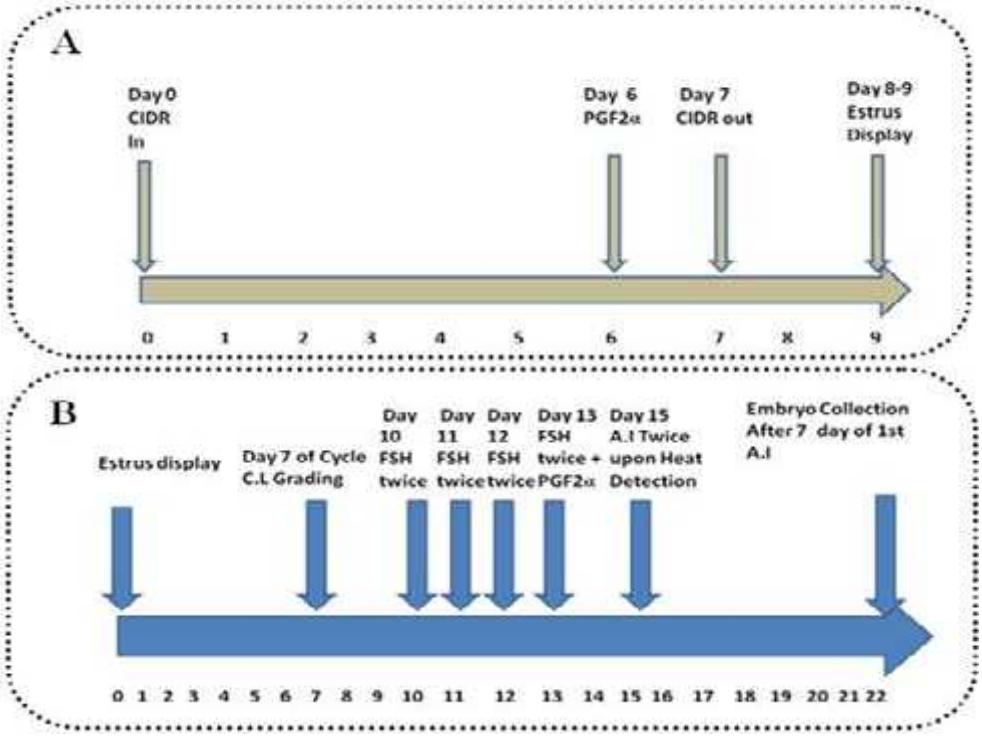

Figure 1. Timeline for, (A) CIDR protocol, (B) super ovulation & embryo recovery.

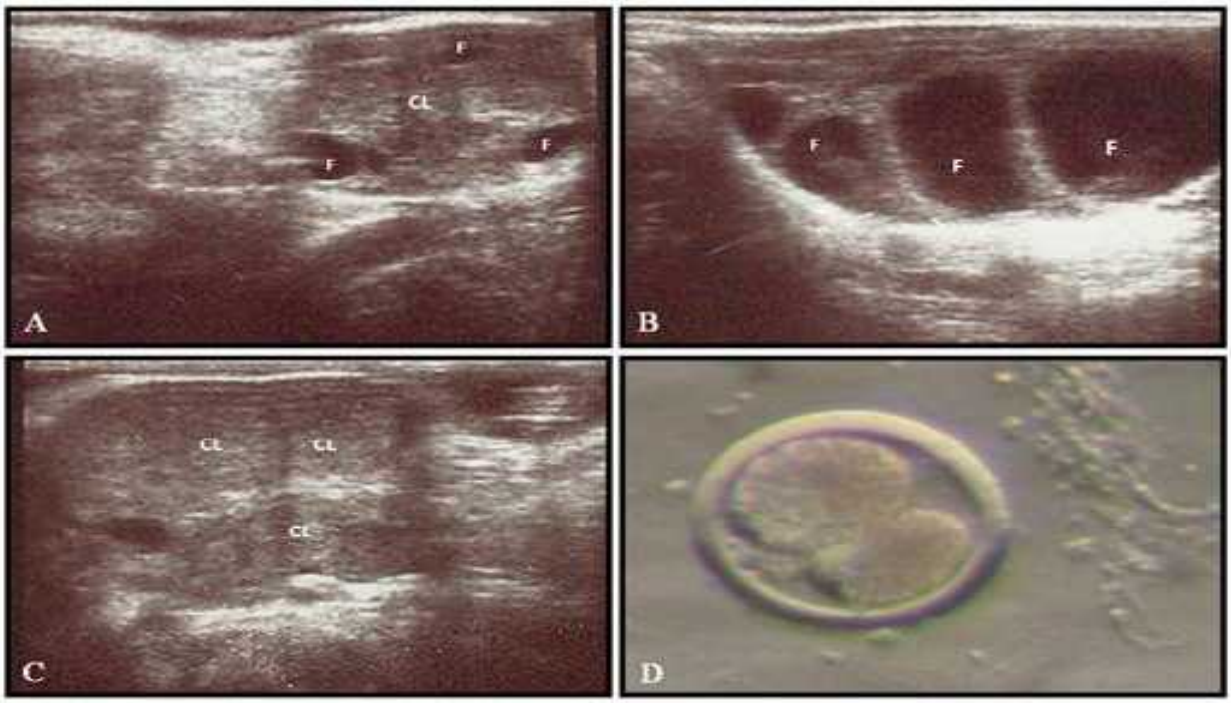

Figure 2. Ultrasound scans of ovaries in cyclicity resumed summer anestrus animal after CIDR exposure.
Ovarian scan of buffalo ovary (A) on day 10 of estrus cycle (before ovarian super stimulation) (B) on day 14 of estrous cycle (after ovarian super simulation) (C) 7 days after heat signs (showing 3 well developed corpora lutea) (D) embryo recovery after flushing the cattle. F represents the follicle, CL represent the corpus lutem.

In experimental stage two, follicular dynamics in response to ovarian stimulation by FSH-p was studied (Table 2). Second follicular wave of the estrous cycle was targeted to harvest, so the FSH injection was started on day 10 of the estrous cycle. Numbers of total follicles in both ovaries were higher in cattle as compared to buffalo (12 vs 7 respectively) on day 10 of estrous cycle. In both species, higher number of follicles was observed in right side ovary as compared to left side ovary. The follicular development pattern was similar in both species. Establishment of follicular dominance was successfully countered by FSH. All of the follicles were smaller than 9mm in diameter. At the end, on day of estrus, number of follicles with more than 9 mm diameter was six in cattle and three in buffalo ovaries. On estrus day, average follicular size was 12mm in cattle, and 10mm in buffalo (Table2). Heat signs with plenty of mucus discharge were observed 36 hrs. after $PGF_{2\alpha}$ injection.

For embryo recovery rate, embryos were recovered on day 7 after AI. Four embryos were recovered from cattle. Only one embryo was of B grade while other three embryos were degenerated and of poor quality (Table3). The embryos were at early embryonic stages than normal development. In buffalo no embryo was recovered due to deformity in the structure of cervix. On ultrasound scanning, four corpora lutea were scanned in cattle and three corpora lutea were scanned in buffalo. The average Corpus luteum size in cattle was 16mm and in buffalo was 12mm.

Table 1. Events regarding the ovarian cyclicity resumption after progesterone (CIDR) exposure to summer anestrus animals.

Species	Estrus after CIDR (hrs)	Duration of Estrus (hrs)	Estrus Intensity (1-3)	Time of ovulation (hrs)	C.L size after ovulation (mm)		Next Cycle	Remarks
					3 days	5 days		
Cattle	36	30	3	38	13	14	+ive	Selected for further protocol
	60	30	2	delayed ovulation	NIL	10	+ive	Rejected for further protocol
Buffalo	36	24	3	41	10	12	+ive	Selected for further protocol
	No estrus	No estrus	1	N.A.	N.A	N.A.	-ive	Rejected for further protocol

Table 2. Follicular development after ovarian super stimulation with FSH-p

Day of estrus	FSH-p dose (armour units)	No. of Follicles											
		Cattle						Buffalo					
		Right Ovary			Left Ovary			Right Ovary			Left Ovary		
		S	M	L	S	M	L	S	M	L	S	M	L
10th (am)	5	6	2	0	4	0	0	4	1	0	3	0	0
10th (pm)	5	6	2	0	3	1	0	4	1	0	3	1	0
11th (am)	5	6	3	0	3	1	0	3	2	1	3	1	0
11th (pm)	5	5	4	2	3	2	1	3	2	1	3	3	0
12th (am)	5	4	4	2	2	3	1	3	2	1	2	3	1
12th (pm)	5	3	6	3	2	3	2	3	3	2	2	3	1
13th (am)	5	3	6	4	2	4	2	2	3	2	2	4	1
13th (pm)	5	2	6	4	2	4	2	2	4	2	2	4	1

S= Small size follicle (≤ 4mm), M= Medium size follicle (5-9mm), L= Large size follicle (> 9mm).

Table 3. Viability status of the embryo recovered

	Total recovered	A - grade	B - grade	Degenerated
Cattle	4	Nil	1	3
Buffalo	Nil	Nil	Nil	Nil

Embryo evaluation criteria

A-grade: Embryos with even granulation and well-defined distinct outline

B grade: Embryo with intact but hazy outline having extruded cells and some degenerate blastomere

Degenerated: Embryos with degenerated blastomeres and not possible to determine the exact developmental stage

DISCUSSION

In Pakistan during summer season conception rate in buffalos is negligible and mostly anestrus behavior is observed (TerMeulen *et al*., 1995). To overcome this problem different estrus synchronization protocols are in practice (Hattab *et al*., 2000; Neglia *et al*., 2003). CIDR protocol is being used to improve fertility rates in ruminants (Cetin *et al*., 2009; Ozyurtlu *et al*., 2011). In this study CIDR application successfully resume ovarian cyclicity. CIDR is a progesterone device having porous membrane that releases P4 in controlled fashion. Whitlock *et al.* (2008) reported that in ovariectomized cows P4 treatment improves KiSSpeptine-10 secretion. It is recently reported that KiSS-peptine is important to improve the pre-ovulatory secretion of LH and GnRH (Smith *et al*., 2011). Hence, the released GnRH after CIDR removal effectively stimulates the pituitary gonadotropins with subsequent estrus induction in anestrus buffaloes. These results strengthen our previous reports that the treatment of P4 could be utilized to improve estrus expression and conception rate in anestrus animals (Naseer *et al*., 2011).

In second stage of the experiment, superovulatory response by FSH was reviewed in these estrous resumed animals. Usually Superovulation treatments are initiated between day 8 to 12 of the estrous cycle, where day 0 is estrus day. These times were originally based on the theory that a wave of follicles in the ovary was maturing at that time (Mapletoft *et al*., 2002). We selected day 10 of the estrous cycle to start ovarian stimulation and studied the follicular dynamics. Our date confirms the previous studies that the exogenous administration of FSH improves the number of dominant follicles development. Our results indicate that in cattle number of dominant follicles developed is higher in number as compared to buffalo. The average follicular size of dominant follicle is also bigger in cattle (12.38±1.4) than buffalo 11.3±0.5.

In this experiment superovulatory response was evaluated by number of corpora lutea formed on Day 7 after the oestrus, using real time, transrectal ultrasonography. In our study, 3-5 corpora lutea were observed. These numbers were less than already reported in buffaloes (Carvalho *et al*., 2002) and cattle (Bo *et al*., 2011). Embryos were recovered 7 days after oestrus but of poor quality. One of the reasons behind poor quality embryo recovery was the effect of season. Summer season directly affects the embryo recovery. The number of embryos decreases in summer as compare to fall season (Manjunatha *et al*., 2009).

Additional factor contributing to low ovarian response and failure to recover embryos in superovulated buffalo are unavailability of homologous FSH (Madan, 1990), incapability of fimbriae to envelope the ovary, low recovery of infused fluid , and faster tubal movement of the embryos (Sharifuddin and Jainuddin, 1984).

However, the number of animals used in the present study was too small to draw any conclusion. But it can be concluded that CIDR protocol, which is steroidal and safe approach for estrus induction and cyclicity in summer anestrus animal. Also superovulation with FSH-p is responsible for the growth of medium size follicles. At the time of embryos recovery presence of number of Corpora lutea in dairy animal are encourage able irrespective of poor quality embryo.

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