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Assessment of Estrus Response and Fertility Rate in Thari Cows Following Estrus Synchronization During Peak and Low Breeding Season

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Authors' Contribution
SAW and AAM designed the experiments. SAW, QK and AK performed the experiments. Data were analyzed by MFH, HS, SK, AS and MJ contributed in reagents/ materials preparation. SAW, AAM and Q wrote the paper.

Key words
Thari Cattle, Estrus synchronization, Estrus response, Fertility rate, Different seasons

ABSTRACT

To investigate the influence of estrus synchronization protocols following ovsynch and ovsynch + CIDR in Thari cows during different breeding seasons. Total (n=80) Thari cows 1st to 4th parity were selected for this study. The experiment was conducted twice in a year i.e. in October to January, n=40 (Peak breeding season) and March to May, n=40 (Low breeding season). Animals were divided into three groups (A, B and C). In group A, 02ml I/M injection of GnRH (25µg lecirelin acetate) were injected on day 0 and 9th of the treatment. On 7th day 05ml I/M injection of PGF2α analogue (5mg/ml dinoprost tromethamine) was administered. The group B animals received same treatment but Controlled Internal Drug Releasing Device (CIDR) (containing 1.38mg progesterone) inserted into vagina on 0 day of the treatment and removed on 7th day of the treatment. Group C (Control group) received 02ml normal saline on 0, 7th and 9th day of the treatment. The positivity of estrus in peak and low breeding seasons was higher in Group B (88.88% and 60%) followed by Group A (73.33% and 46.66%) and Group C (14.28% and 10%). Similarly Group B animals showed higher fertility rate (66.66% and 46.66%) followed by Group A (53.33% and 26.66%) and C (00% and 00%) during peak and low breeding seasons. It was concluded that the ovsynch plus CIDR induces superior estrus incidence and fertility as matched to other treatments and control group.

INTRODUCTION

Pakistan is a 4th largest milk producing country in the world, but the reproductive performance of dairy animals is generally affected by summer conditions as we know that mostly non-cyclic status of the animals is documented during that season, estrus synchronization is one of the solutions to the reduced anestrous state (Kalwar *et al*., 2016). In Pakistan animals with altered genetic potential are available (ranging from 2 liters to 25 liters daily milk yield) (Afzal and Naqvi, 2004). Pakistan consists of various well-known breeds, i.e., Thari, Lohani, Red Sindhi, Sahiwal, Cholistani, Bhagnari, Rojhan, Dhanni, and Dajal. They can survive very well in humid and hot climate conditions. Amongst these cattle breeds, Thari cattle is an important breed that shares 23% of the total cattle population in Sindh province (Hussain *et al*., 2014). This cattle breed is found in the India-Pakistan border area and is well-thought-out as the good draught and dairy animal of subtropics and tropics (Shahani, 1991). Thari cows are medium-sized animals with a long tapering face, a little convex forehead, horns are medium sized that curved upward and outward and large semi-pendulous ears have a well-developed firm hump, medium dewlap, and strong legs. The tail switch is black; udder is large and strong, adult males and females weight 400-500 and 300-380 kg, respectively (Khan and Esani, 1994).

Estrus synchronization (ES) is a vital management tool for the enhancement of productivity. In estrus synchronization, the group of females is brought into

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estrus (heat) at a predetermined time (Attyia and Abdelmoneim, 2014). Timed artificial insemination (TAI) and estrus synchronization improve pregnancy rate. GnRH, prostaglandin F2α, progesterone, and estrogen hormones are used in estrus synchronization (Pursley *et al*., 1995). PGF2α is a naturally occurring hormone. It is released from the uterus on day 16 to 18 of a non-pregnant animal during normal estrus cycle and causes leutolysis of corpus luteum (CL) (Abecia *et al*., 2011). Due to the destruction of CL, a new reproductive cycle is started during which animal displayed estrus behavior.

Gonadotropin releasing hormone (GnRH) is synthesized in the cytoplasm of the diencephalon and packaged to move into the Golgi apparatus (Huirne and Lambalk, 2001). Progesterone concentrations decreased in the blood stream if the CL is regressed, and causing a rise in GnRH concentration. This upsurge of GnRH allows for high pulsatile secretions of LH and FSH. FSH is released from the pituitary and acts on the ovary. It helps to stimulate follicular waves. LH is released from the pituitary in a surge and acts on the dominant follicle to prepare them for ovulation approximately 24 h before actual ovulation (Rahman *et al.,* 2008). By the action of CL progesterone prevents the expression of estrus. Progesterone act to prevent the LH surge and thereby prevent ovulation. An additional benefit of using progesterone is that they can initiate estrus cycles in anestrus cows and pre-pubertal heifers. Progesterone can be used into various routes, i.e., parental, local, intra-vaginal and oral. The two most common routes of progesterone administration are intra-vaginal and oral.

Addition of progesterone in the ES procedure prohibits the onset of early estrus and a corpus lutum with average lifespan was produced following progesterone with drawal. Several studies reported that the addition of progesterone in an ES practice results suppress estrus and synchronize ovulation, thus allowing a higher submission rate for TAI without disturbing fertility.

Very little work has been done to assess the effectiveness of various synchronization protocols in indigenous breeds of cattle in Sindh. Synchronization of estrus is a very useful tool for extensive use of artificial insemination in scattered livestock population in Thari cows. However, it is necessary to evaluate the efficacy of various estrus synchronization protocols in terms of timing of heat and conception rate at synchronized heat before application of the technique in the field. Therefore, the current study was planned in Thari cows to examine the effect of various estrus synchronization protocols.

MATERIALS AND METHODS

Animals and management

The study was conducted on 80 Thari cows 1st to 4th parity maintained on semi-intensive management conditions at Thari Cattle Farm Nabisar Road, Distt: Umerkot and its surroundings. The animals with good reproductive health (farm record) were incorporated in the current reasearch. Prior to the start of experiment all the animals were confirmed to be nonpregnant through rectal palpation. The regular feeding and drinking practices were observed during the experimental period.

Experimental design

Animals were divided into three groups (A, B and C). The experiment was conducted twice a year, i.e. in October to January, n=40 (Peak breeding season) and March to May, n=40 (Low breeding season).

Group A were given 2ml (IM injection) of GnRH analogue (25µg lecirelin acetate; Delmarelin®, FATRO Italy) on 0 day of the treatment. On 7th day PGF2α analogue 5ml (5mg/ml dinoprost tromethamine salt; Lutalyse™, Pfizer, Belgium) was administered and a second 2ml injection of GnRH analogue was given on the 9th day of the treatment.

Group B received 2ml of GnRH analogue (25µg lecirelin acetate; Delmarelin®, FATRO Italy) and Controlled Internal Drug Releasing Device (CIDR) inserted into the vagina (containing 1.38mg progesterone, EAZI-BREED™ CIDR®, New Zealand) on 0 day of the treatment. On 7th day animals were given PGF2α analogue 05ml (5mg/ml mg dinoprost tromethamine salt; Lutalyse™, Pfizer, Belgium) and on the same day CIDR was removed. After the 48 hours of injection of PGF2α, the next dose of GnRH injection was given to the animals.

Group C (Control group) were injected 2ml normal saline on 0, 7th and 9th day of the treatment.

Determination of heat and artificial insemination

Experimental animals were observed from the start of the experiment until the end for behavioral changes to check the estrus. The estrus intensity was categorized as (i) Strong when the animals exhibited mounting behavior and bellowing along with other genital changes such like the discharge of mucus from the vagina, congested vaginal mucus membrane, swollen of vulva and vagina, and increased regularity of micturition were classified as a strong response. (ii) Moderate when animal had discharge of mucus from the vagina, mucus membrane of vagina congested, edematous swollen vulva and vagina and increased frequency of micturition. (iii) Weak when the animal showed some congestion in vaginal mucus membrane.

Estrus signs were noted for half an hour each time at 6 am, 12 pm, 6 pm by visual examination. Beginning and cessation of estrus, especially stand to be mounted were recorded for every Thari cow for estrus signs.

Artificial insemination was performed two times at 12 and 24 h after the last injection in all treatment groups with frozen-thawed semen obtained from Directorate of Animal Breeding, Livestock Department, Govt. of Sindh.

Pregnancy diagnosis

Pregnancy diagnoses were conducted at day 60 post-AI through rectal palpation.

Statistical analysis

This data was composed and analyzed by ANOVA using 8.1 versions.

RESULTS

This research was planned to assess the effect of two estrous synchronization protocols such as ovsynch and ovsynch + CIDR protocols in Thari cows during peak and low breeding seasons.

Estrus response

The effects of ES on the incidence of estrus in various groups of animals in peak and low breeding seasons are summarized in the Table I. Animals of group B showed higher response of estrus (88.88% and 60%) than that of group A (73.33% and 46.66%) and group C (14.28% and 10%) during peak and low breeding seasons.

The effects of ES protocols ovsynch and ovsynch plus CIDR on estrus response in primiparious and multiparious Thari cows during peak and low breeding seasons are shown in Table II. Primiparious and multiparious animals of group B during both breeding seasons showed significantly ($P<0.05$) higher estrus response as compared to primiparious and multiparious animals of groups A and C.

Onset of estrus

The effects of ES protocols ovsynch and ovsynch plus CIDR on the onset of estrus in different groups of Thari cows during peak and low breeding seasons are summarized in Table I. During both seasons, nonsignificant ($P>0.05$) difference was observed between the group A (47.727 ± 4.361 h, 47.714 ± 1.113 h), B (45.188 ±2.455 h, 46.000 ± 9.992 h), and C (56 h, 57 h). Furthermore, Group B animals display earlier estrus as compared to group A and C during peak and low breeding seasons.

Duration of estrus

The effect of ES protocols ovsynch and ovsynch plus CIDR on the duration of estrus in various groups of Thari cows during peak and low breeding seasons are concise in Table I. During both seasons significant difference ($P<0.05$) were detected between the group A (22.364 ± 2.420 h, 21.857 ± 1.069 h), B (24.500 ± 2.066 h, 24.222 ± 1.093, h), and C group (21 h, 20 h) for duration of estrus in peak and low breeding seasons. Furthermore, group B animals remained maximum time in estrus as compared to group A and C during peak and low breeding seasons.

Intensity of estrus

The effects of ES protocols (ovsynch and ovsynch plus CIDR) on the intensity of estrus during peak and low breeding seasons in Thari cows are show in Table II. Here was significant different ($P< 0.05$) were observed within the groups. Higher number of group B animals showed strong estrus signs than group A and C in peak and low breeding seasons.

Fertility rate

The effects of estrus synchronization protocols (ovsynch and ovsynch plus CIDR) on the fertility rate in different groups of Thari cows during peak and low breeding seasons are shown in Table I. Non-significant ($P>0.05$) difference observed between the groups for

Table I. Effect of estrus synchronization protocols (ovsynch and ovsynch plus CIDR) on estrus response, onset of estrus, duration of estrus and on fertility rate in Thari Cows during peak and low breeding seasons.

Treatment	Control (n=17)	Ovsynch (n=30)	Ovsynch+ CIDR (n=33)	P value
Estrus response (%)				
Peak breeding season	01/07 (14.28%)	11/15 (73.33%)	16/18 (88.88%)	0.043
Low breeding season	01/10 (10%)	07/15 (46.66%)	09/15 (60.00%)	
Onset of estrus (h)				
Peak breeding season	(56.00 h)	(47.727 ± 4.361 h)	(45.188 ± 2.455 h)	0.0541
Low breeding season	(57.00 h)	(47.714 ± 1.113 h)	(46.000 ± 9.992 h)	0.064
Duration of estrus (h)				
Peak breeding season	(21.00 h)	22.364 ± 2.420 h)	(24.500 ± 2.066 h)	0.0177
Low breeding season	(20.00 h)	(21.857 ± 1.069 h)	(24.222 ± 1.093 h)	0.0015
Fertility rate (%)				
Peak breeding season	00/07 (00%)	08/15 (53.33%) 04/15	12/18 (66.66%)	0.0573
Low breeding season	00/10 (00%)	(26.66%)	07/15 (46.66%)	

Table II. Effect of estrus synchronization protocols (ovsynch and ovsynch plus CIDR) estrus response, intensity of estrus, and on fertility rate in different parity of Thari cows during peak and low breeding seasons.

Treatment	Control (n=17)		Ovsynch(n=30)		Ovsynch+ CIDR (n=33)	
	Primiparious	Multiparious	Primiparious	Multiparious	Primiparious	Multiparious
Estrus response (%)						
Peak breeding season	0/3 (00%)	1/4 (25%)	3/5 (60%)	8/10 (80%)	4/5 (80%)	12/13 (92%)
Low breeding season	0/5 (00%)	1/5 (20%)	2/5 (40%)	5/10 (50%)	3/5 (60%)	6/10 (60%)
Intensity of estrus (%)						
Peak breeding season						
Strong (%)	0/3 (00%)	1/4 (25%)	2/5 (40%)	4/10 (40%)	3/10 (60%)	7/13 (53%)
Moderate (%)	0/3 (00%)	0/4 (00%)	1/5 (20%)	3/10 (30%)	1/5 (20%)	5/13 (38%)
Weak (%)	0/3 (00%)	0/4 (00%)	0/5 (00%)	1/10 (10%)	0/5 (00%)	0/13 (00%)
Low breeding season						
Strong (%)	0/5 (00%)	1/5 (20%)	1/5 (20%)	2/10 (20%)	3/10 (60%)	4/10 (40%)
Moderate (%)	0/5 (00%)	0/5 (00%)	1/5 (20%)	2/10(20%)	0/10 (00%)	2/10 (20%)
Weak (%)	0/5 (00%)	0/5 (00%)	0/5 (00%)	1/10 (10%)	0/10 (00%)	0/10 (00%)
Fertility rate (%)						
Peak breeding season	0/3 (00%)	0/4 (00%)	2/5 (40%)	6/10 (60%)	3/5 (60%)	9/13 (75%)
Low breeding season	0/5 (00%)	0/5 (00%)	1/5 (20%)	3/10 (30%)	3/5 (60%)	4/10 (40%)

fertility rate in group A (53.33%, 26.66%), B (66.66%, 46.66%), and C group (00%, 00%). Higher fertility rate observed in group B than group A and C during peak and low breeding seasons.

The effect of estrus synchronization protocols in ovsynch and ovsynch plus CIDR on fertility rate in different parity of Thari cows during peak and low breeding seasons are show in Table II. There was statistically significant ($P < 0.05$) difference in the fertility rate in different parity. Primiparious and multiparious animals of group B showed better fertility rate in peak and low breeding seasons than the primiparious and multiparious animals of group A and C.

DISCUSSION

Summer anestrus is one of the significant problem in the dairy animals of subtropical and tropical countries. During summer, the majority of the animals are in anestrus condition. Many estrus synchronization protocols have been studied they can be reduce these problems. The ovsynch protocol is a series of GnRH-PGF2α-GnRH treatments that became accepted for estrus synchronization in cattle over the last decade, resulting in an acceptable fertility to timed AI (TAI) (Jabeen *et al*., 2012). In the present study, ovsynch protocol induces estrus during peak in 73% and low breeding season in 46.66% Thari cows. Results of this study agree with those of Jabeen *et al*. (2012) who reported 87.5% estrus response during peak breeding season and 36.36% during low breeding season in buffaloes with ovsynch protocol. Similarly, Warraich *et al*. (2008) reported 62.5% estrus response during peak breeding season and 55.5% estrus response during low breeding season in Nili Ravi buffaloes. The difference in results may be due to how estrus was observed, type of housing, frequency of handling, environmental condition etc.

Ovsynch plus CIDR protocol animals showed estrus response 88% during peak breeding season and 60% during low breeding season. The results of present study are higher than Ravi-Kumar *et al*. (2007) and Singh *et al*. (2010) described 58.33% and 37.5% estrus response with CIDR in buffaloes, respectively. While current results are lower than Yotov *et al*. (2012) who reported significantly ($P<0.05$) higher (94.4%) estrus response with PRID based treatment than ovsynch (66.7%). Similarly, Virmani *et al*. (2013) obtained higher estrus response than present study. They determined the efficacy of different hormonal protocols in postpartum anestrus sahiwal cows. The difference in the results may be due to environmental conditions, nutritional conditions, body conditions and temperature.

During both seasons in ovsynch and ovsynch plus CIDR protocol multiparious animals showed significantly ($P <0.05$) better estrus response as compared to primiparious animals. Similarly, Wathes *et al*. (2001) reported better estrus response in multiparious animals as compared to primiparious animals. Results of current study are different from those of Moreira *et al*. (2001) and Bousquet *et al*. (2004) who observed superior estrus response in primiparous cows than multiparous cows. During peak and low breeding seasons ovsynch plus CIDR protocol animals came earlier in estrus (45.188 ± 2.455 h, 46.000 ± 9.992 h) than ovsynch protocol (47.727 ± 4.361

h, 47.714 ± 1.113 h) and control (56 h, 57 h). Similarly ovsynch plus CIDR group animals remained in estrus for maximum time (24.500 ± 2.066 h, 24.222 ± 1.093 h) as compared with ovsynch (22.364 ± 2.420 h, 21.857 ± 1.069 h) and control (21h, 20h). Likewise onset of estrus was observed earlier in ovsynch plus CIDR group than other groups. Sahatpure and Patil (2008) reported 54.50±2.60 h and 21.25 ± 0.881h for duration of estrus in non-descript cows.

Higher estrus intensity was observed in peak breeding season as compared to low breeding season. Furthermore, intense estrus were observed (60%, 53% and 60%, 40%) in ovsynch plus CIDR group than ovsynch (40%,40% and 20%, 20%) and control group (00%,25% and 00%,20%). Results of Ravi-Kumar *et al*. (2007) are in contrast with those of present results who reported 18.18% strong estrus signs and 63.63% moderate estrus signs in animals with ovsynch protocol. Similarly, Singh *et al*. (2003) agreed with present results and reported that (23.91%) strong, (26.08%) moderate and (15.21%) week estrus signs, respectively.

In the present study 53% fertility rate was observed with ovsynch protocol during peak breeding season and 27% during the low breeding season. The conclusion of the current study is better than the result of Baruselli et al. (2003) who reported 48.8% fertility rate in peak breeding season and 6.9% during the low breeding season in buffaloes. Our findings are lower than those of Warraich *et al*. (2008) who reported 30.4% fertility rate in low breeding season. The findings of present study are also higher than the Rensis *et al*. (2005) they reported extremely low conception rate (4.7%) in buffaloes with ovsynch protocol during low breeding season.

In this study 66.66% fertility rate was observed with ovsynch+CIDR protocol in Thari cows during peak breeding season and 46.66% during the low breeding season. These findings are higher during breeding season than the result of Stevenson *et al*. (2006) who reported 50% fertility rate in dairy cows with ovsynch plus CIDR protocol. A similar pattern of fertility rate was reported by Abdallah and Rahim (2014) who reported 36.1% fertility rate in cows with ovsynch plus CIDR protocol. The findings of current study are lower than the findings of Attyia and Abdelmoneim (2014) who observed 80% fertility rate with ovsynch plus CIDR protocol in non-cyclic cows.

In present results multiparious animals showed significantly ($P < 0.05$) greater fertility rate as compared to primiparious animals in peak and low breeding seasons in both ovsynch and ovsynch plus CIDR protocol. Similarly, Mwaanga *et al*. (2012) and Wathes *et al*. (2001) also evaluated that multiparious animals showed greater fertility rate as compared to primariparious animals. Our results are consistent with those of Tenhagen *et al*. (2003) and Bousquet *et al*. (2004) who observed higher fertility rate in primiparous cows than in multiparous cows. The variation in the outcomes may be due to housing management, environmental conditions or body conditions.

CONCLUSION

Present study indicates that the ovsynch plus CIDR group induces better estrus response and fertility rate in both seasons as compared to ovsynch and control group. So, ovsynch plus CIDR can effectively be used to induce cyclic activities and increases fertility rate and estrus response in Thari cows.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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