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Efficacy of ovsynch protocol on ovarian response and progesterone profile in yak

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The reproductive performance in yaks *(Poephagus grunniens* L.) is known to suffer from several inherent problems like late maturity, poor estrus expression, distinct seasonal reproductive patterns, prolonged intercalving intervals and low conception rates. Besides these, silent estrus and inability to detect estrus are other major limitations in yak reproduction (Sarkar and Prakash 2005). Therefore, application of reproductive biotechniques like artificial insemination (A.I.) in yaks is not easier as in cattle. Considerable research has long been made with an attempt to enhance its reproductive efficiency in yak rearing countries of the world. Synchronization of estrus and ovulation and fixed- time AI could be a solution to over come the difficulty of oestrus detection in yaks. The ovsynch protocol (administration of GnRH, followed by PGF2α 7 days later and a second GnRH 2 day later after PGF2α) is easier and less expensive than protocols based on exogenous progesterone (Neglia *et al*. 2003). The protocol induced ovulation in 100% non-cyclic Egyptian buffalo cows in March and April (Ali and Fahmy 2007), which encouraged us to evaluate the effect of ovsynch protocol on ovarian response and progesterone profile in yak cows.

Present study was carried out on 8 female yaks maintained in the institute farm located at 2,750 m above mean sea level during the month of May-June. All the animals were kept under similar management conditions in order to minimize environmental differences; animals were housed inside and fed concentrate mixture composed of maize grain, wheat bran, mustard cake, mineral mixture and salt as per the live body weight. Green fodder was given *ad lib.* to meet the nutritional requirements. The study group gave normal parturition and had no history of reproductive disorders. Each animal was treated as per ovsynch protocol consisting of 10 µg GnRH analogue i.m. at days 0 and 9 and 500 µg synthetic PGF_2 i.m. at day 7. The ovsynch protocol was followed regardless of the stage of estrous cycle in the female yaks. All yaks were inseminated 16–20 h after the second GnRH administration, with frozen –thawed semen from yak bulls of known high fertility. The pregnancy diagnosis was carried out at 45 days post A.I. by per rectal examination.

Ultrasound scanning of the ovaries was carried out in all the animals by the same operator using a real time B-mode ultrasound scanner equipped with 7.5 MHz linear –array rectal transducer daily from day 0 (before administration of GnRH) to day 10. Follicles ≥ 3 mm in diameter and the corpus luteum (CL) were measured and recorded. After each scan, a sketch was made of each ovary for subsequent evaluation. Ovulation was considered to have occurred when a large growing antral follicle that had been identified and followed for several days was no longer observed. Emergence of follicular wave was defined as occurring on the day on which the retrospectively identified dominant follicle was 4 mm (Ginther *et al*. 1997). Luteal regression following PGF2α treatment was considered when progesterone concentration was <1 ng/ml. Follicle luteinization was considered when a follicle did not ovulate; instead luteal tissue gradually developed and was initially detected as echogenic particles scattered inside the anechoic antrum. Blood samples were collected daily from jugular vein of each animal immediately after ultrasound scanning and plasma was separated and stored at –20°C until progesterone (P_4) analysis. The P_4 concentration in plasma was determined following radio-immunoassay using progesterone C.T. RIA kits. A blood P_4 value ≥ 0.5 ng/ml was considered to be an indicator of the presence of functional CL (Repasi *et al*. 2005). The intra-assay and inter-assay coefficient of variance were 5.4 and 8.6% respectively.

The response rates to the first GnRH (by ovulation or luteinzation) were recoded in all the yaks. In 7 out of 8 animals (87.5%) there is ovulation and in 1 animal (12.5%) luteinzation of the follicle was seen. The average diameter of the follicles responding to the first GnRH injection was

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recorded to be 9.20±0.35 mm. As a result of administration of the first GnRH, a new follicular wave emerged followed by development of a new dominant follicle in all the yaks. The first GnRH was designed to ovulate large functional follicles and to induce a new follicular wave (Wiltbank 1998). The second rationale was to increase the percentage of animals synchronised to a single injection of PGF2α (Ali and Fahmy 2007). Ovulation of the dominant follicle occurred in 87.5% animals. Similarly, ovulation rate of approximately 86% was recorded in cyclic buffaloes (de Araujo Berber *et al.* 2005) and 82–90% in cyclic cattle (Wiltbank 1998). The first GnRH was also successful in synchronising a new follicular wave 1–3 days after treatment. Similarly, Ali and Fahmy (2007) also recorded development of follicular waves in buffaloes within day 1–3 of treatment. These waves result in the development of a new dominant follicle in all the treated animals.

By day 7, a follicle larger than 8 mm in diameter was detected in 62.5% of the yaks. The overall mean diameter of the follicles on day 7 was recorded to be 8.84±0.85 mm. A mature CL was seen in all the yaks on the seventh day. The overall mean diameter of the CL was 9.80±1.05 mm. Luteolytic responses to $PGF_2\alpha$ treatment were recorded in all the animals. There was a considerable decrease in the size of CL form day 7 to day 8 and 9. The dominant follicle increases in size and the average mean diameter of the dominant follicle on day 9 was 10.81±0.52 mm. This high synchrony between animals; presence of functional CL and a large active follicle at the same time were due to the first GnRH treatment (Ali and Fahmy 2007). After the PGF2α injection, the CL regressed efficiently in all the animals, indicating the maturity of the CL by this time. Similar result indicating 95% regression in cattle was recorded by Wiltbank (1998).

Injection of second GnRH resulted ovulation in 75% (6 of 8) of the yaks within 24h of the administration. In the animals that ovulated oestrul mucus was observed in four animals on the next day of the second GnRH treatment. To increase synchrony of ovulation, a second GnRH was injected to ovulate the preovulatory follicle at the precise time (Wiltbank 1998). Present findings were similar to earlier reports in cyclic cows (81.1%) by Karen and Darwish (2010). Ali and Fahmy (2007) reported ovulation in 80% cyclic and 100% in acyclic buffalo cows. The difference may be due to the stage of oestrus cycle in which protocol was initiated, season of year and the number of animals in the trial.

The mean P_4 level on day 7 was 3.01±0.21 ng/ml. After administration of PGF2α on day 7 the mean blood P_4 concentration declined significantly ($P<0.05$) on day 9 (0.33±0.19 ng/ml). On the day of AI the mean concentration level of blood P_4 was 0.19±0.04 ng/ml. Sarkar *et al.* (2005) also reported that the concentration of plasma P_4 in yak reached to basal level (≤ 0.2 ng/ml) at estrus, which was similar to the present findings.

Table 1. Ovarian responses to ovsynch protocol in yak

Ovarian responses	Yaks (n=8)
Ovarian finding at day 0	
Follicular size (mm)	9.20±0.35
F >8 mm (n)	5/8
CL (n)	3/8
Response to first GnRH	
Ovulation (n)	7/8
Luteinization (n)	1/8
Follicular waves	8/8
Emergence of follicular waves (days)	1–3
Ovarian findings at day 7	
CL (n)	8/8
CL size (mm)	9.80±1.05
Follicular size (mm)	8.84±0.85
F> 8 mm (n)	5/8
Ovarian findings at day 9	
Luteolysis (n)	8/8
F > 8 mm (n)	8/8
Follicular size (mm)	10.81±0.52
Response to second GnRH	
Ovulation (within 24 h)	6/8
Cervical mucus noticed (n)	4/6
Conception rate (n)	1/8

The overall conception rate (CR) recorded in the present experiment was 12.5% (1 of 8). Karen and Darwish (2010) reported CR of 18% (2 of 11) and 0% (0 of 10) in cyclic and non-cyclic buffalo cows respectively in summer. Many other factors related to handling of semen and inseminators may contribute to lower conception rate in the present experiment.

To summarize, the yaks responded efficiently to different steps of ovsynch protocol as evident by the ultrasonic ovarian examination and plasma progesterone analysis. However, overall CR obtained in the present study was comparatively low; hence trials involving more animals with suitable modification of the protocol may be undertaken, thereby making the protocol beneficial for fixed time A.I. and to overcome oestrus detection problems in yaks.

SUMMARY

Efficacy of ovsynch protocol in yaks was studied in 8 animals. Ovarian ultrasound to study the ovarian response and blood sampling for progesterone analysis was performed daily. The follicles and CL diameter were measured and recorded. The first GnRH administration resulted in either ovulation or luteinisation of follicles in all the animals. By day 7 almost all animals had a large follicle and lutein tissue. Luteolytic response to PGF_2 was observed in all the animals. Following second GnRH, ovulation occurs within 24h in 6 animals. Administration of PGF_2 (day 7) resulted in significant reduction of plasma progesterone concentration at day 9.

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