

Original Research Article

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## Synchronization of Estrus using Prostaglandins and HcG in Indigenous Mares during Transitional Period

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### ABSTRACT

#### Keywords

Marwari mares,  
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The experiment was conducted on 20 Marwari mares of age between 4-10 years, during transitional period (January - March). They were randomly divided into two groups. In group 1 (control group; n=10) ovarian ultrasonography was done using linear array transducer to detect dominant follicle ( $\geq 30$ mm) on any of the ovaries in mares showing estrus signs. Mares were covered twice after 36 hours interval. In group 2 (treatment group; n=10) ultrasonography was done for detection of ovarian status. Mares with mature corpus luteum (CL) and growing follicle between 18mm-24mm in size were synchronized with hormonal protocol based on prostaglandin (PGs 500  $\mu$ g) and Human chorionic gonadotropin (HcG 3000 I.U.). Day of detection of mature CL was considered as day 1. On day 1 morning mares were administered PG intramuscular followed by administration of chorulon intravenously on 6<sup>th</sup> day morning and after 8 days again PG intramuscular on day 14<sup>th</sup> morning followed by chorulon intravenously on 20<sup>th</sup> day morning. Mares were covered twice after 36 hours of interval between 12-48 hours post last chorulon injection. It was concluded that during transition period the incidence of large dominant follicles regression without ovulation is very high and conception rates are very low (40%). The application of exogenous hormones to monitor normal estrus length and ovulation is quite effective during transition period. Hormones protocols based on Prostaglandins and HcG will leads to successfully regulates normal length of estrus and ovulation in indigenous mares and high conception rates (70 %) are achieved.

### Introduction

The Marwari mares come under the category of long day breeders among the seasonal breeder animals. In tropical countries including India, there is onset of oestrous cyclic activity with increase in day length (photoperiod) and breeding season reaches its peak when day length reach up to 13-14 hours (May to July). The ovaries become

active, soft and large in size (2-3cm Diameter) peak breeding season mares are having regular oestrous cycle length but as the day length starts decreasing the subsequent oestrous cycles start becoming irregular and finally mares enter into anestrus period (November-December). When day length is around 8-9 hours, ovaries become reproductively inactive, smooth small (1-1.5cm diameter) and there are no signs of estrus.

Afterwards as the day length starts increasing (January-March) mares enter into the transitional phase. During this period the ovaries are turning from reproductive inactivity to maximal activity, ovarian follicles start developing normally. But only few mares show estrus signs with prolonged cycle length, dominant follicles may decrease in size and regress without ovulation. Therefore conception rates are very low during transitional period Sertich (2020). Normal estrous cycle starts occurring after 1-1.5 months from first transitional estrous cycle of mare.

Pineal gland is mainly responsible for stimulation of estrous cycle which is responsive to increasing day length intensity (photoperiod) either natural or artificial. During increase in photoperiod there is reduction in melatonin secretion as a result hypothalamus gets stimulated to secrete gonadotropins releasing hormone (GnRh). This is further responsible for releasing sufficient FSH and LH from anterior pituitary Vidauri *et al.*, (2018) and mares turn to normal cyclicity of breeding season.

In India the trend of using exogenous hormones for synchronization of estrus in Marwari mares is very uncommon. Unawareness among breeders about anestrus and transitional period results in increasing incidence of various reproductive disorders like conception failure, embryonic deaths and low conception rates.

## **Materials and Methods**

The experiment was conducted on 20 Marwari mares of age between 4-10 years, during transitional period of breeding from (January-March). The mares with normal previous foaling, irregular estrus length, clear uterine discharge, no history of conception failure and good body condition score (3/5)

were selected for the study. They were randomly divided into two groups.

In group 1 (control group; n=10) ovarian ultrasonography (USG) was done using linear array transducer with 5/7.5 interchangeable frequency (BCF technology) to detect dominant follicle ( $\geq 30$ mm) on any of the ovaries in mares showing estrus signs. Mares were covered twice after 36 hours interval.

In group 2 (treatment group; n=10) ultrasonography was done for detection of ovarian status. Mares with mature corpus luteum (CL) and growing follicle between 18mm-24mm in size were synchronized with hormonal protocol based on prostaglandin (PGs) (Estrumate, cloprostenol sodium 500  $\mu$ g) and Human chorionic gonadotropin (HcG) (Chorulon 3000 I.U.) hormone. Day of detection of mature CL was considered as day 1. On day 1 morning mares were administered with injection estrumate (500 $\mu$ g) intramuscular followed by administration of injection chorulon (HcG 3000 I.U.) intravenously on 6<sup>th</sup> day morning and after 8 days again injection estrumate (500 $\mu$ g) intramuscular on day 14<sup>th</sup> morning followed by injection chorulon 3000 I.U. intravenously on 20<sup>th</sup> day morning. Mares were covered twice after 36 hours of interval between 12-48 hours post last chorulon injection.

After 24 hours of last covering ultrasonography done for the confirmation of ovulation. Pregnancy was detected 30 days from last covering in both the groups.

The numerical data regarding duration of estrus, size of dominant follicle are represented as Mean $\pm$ SEM and differences were considered to be significant at  $P < 0.05$ . The statistical analysis for significant was done using student's t-test and chi square test according to data represented.

## Results and Discussion

In group 1 (n-10) mares during transition period the Mean±SEM duration of estrus was 8.2±0.2 days (Table.1). Ginther (1990) concluded that average occurrence of ovulation was 9.5±0.7 i.e. longer for end of transition phase. Extreme variability in duration of estrus and characterized by the presence of prolonged and irregular estrus of mares along with erratic sexual activity in early transition period Satue and gardon (2013). The Mean±SEM of follicle size in group 1 was 34±0.2mm. Transition period was characterized an ovulatory dominant size of follicle that had growing, static and regressing phases and maximum diameter of follicle may reach greater than 38mm Ginther (1990). During transition period there were occurrence of irregular estrus cycles and high incidence of dominant follicle regression and pregnancy rates of mares was lower (51.5%) from the first ovulation of the season Cuervo-Arango and Clark(2010). The average no. of mares successfully ovulated during transition period and the conception rates were 40% (4/10) and 30% (3/10) respectively.

In group 2 (n-10) artificial control of estrous cycle of mares during transition period by the usage of exogenous hormone protocol. The presence of mature dominant corpus luteum on either side of ovary which acts as a source of progesterone. The first prostaglandin (PGF2 $\alpha$ ) injection was given to remove luteal tissue and falling in progesterone concentration. A single injection PGF2 $\alpha$  is effective to cause luteolysis after 5 days of previous ovulation in mares Douglas and Ginther (1975). In group 2 the mares were arrived to estrus after 48-60 hours of prostaglandins injection. Norman and Larsen(2010), Potter (2017) reported that mares treated with PGF2 $\alpha$  come to estrous within 3-5 days. Mares with confirmed fully

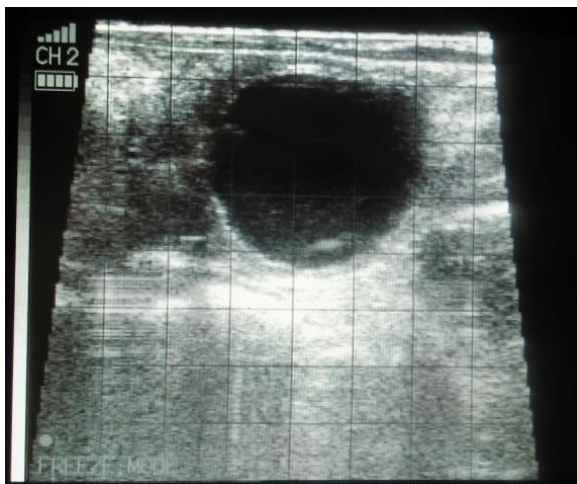
functional corpus luteum, prostaglandins has 80% luteolytic rate at single dose to mares 5 days post ovulation and with the luteolysis of CL the concentration of progesterone decrease drastically. The percentage of mares respond to first PGF2 $\alpha$  on day 1 morning intramuscularly of synchronization was 100% (10/10).The developing follicles at subsequent ovary develop towards dominant and injection HcG 3000 I.U. intravenously during 1<sup>st</sup> cycle had a shorter estrus and resulted into ovulation sooner than group 1 mares (control). The first course of PGF2 $\alpha$  and HcG was designed to remove all luteal tissue and to induce ovulation of the follicles that developed in response to the withdrawal of progesterone Palmer and Jousset (1975). After the successful ovulation during first course the formation of corpus luteum begins and it acquires more than 5 days of next cycle to respond to PGF2 $\alpha$  again. The functional development of corpus luteum reaches after day 5 of ovulation and secretes progesterone up to 4 ng/ml Kelleman (2013).

On day 14<sup>th</sup> morning of the controlled estrous cycle mares were short cycled to estrus by giving PGF2 $\alpha$  after detecting functional corpus luteum by ultrasonographic examination. Larson (2013) reported that the administration of PGF2 $\alpha$  during diestrus induces luteolysis within 24-48 hours and mares will return to estrus within two days post PGF2 $\alpha$  administration. In group 2 mares during 2<sup>nd</sup> course of prostaglandins mares returned to estrus within 48-60 hours with visual signs of estrus like winking of clitoris, tail raising and urinating in response to teasing with the stallion. Mares were regularly examined with USG on alternate days to monitor the developing follicle. Then on day 20<sup>th</sup> morning of controlled estrous cycle mares were administrated with HcG 3000 I.U. intravenously after detection of dominant follicle through USG.

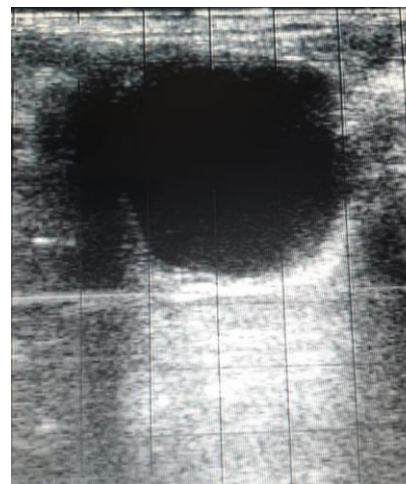
**Table.1** Comparison of both the groups for different observations

S.no	Parameters (Mean ±SEM)	Group 1 ( n-10)	Group 2 ( n-10)
1.	Duration of estrus (days)	8.2 ± 0.2*	6.3 ± 0.1
2.	Dominant follicle size (mm)	34.2 ± 0.2*	31.4 ± 0.2
3.	Ovulation rates	40% (4/10)	80% (8/10)
4.	Conception rates	30% (3/10)*	70% (7/10)

Significant at P<0.05



**Fig.1** Dominant follicle ≥ 31mm after estrus synchronization.



**Fig.2** Regressing follicle ≥ 35mm during natural transition period.



**Fig.3** Natural covering of mare after estrus synchronization.

The Mean±SEM dominant follicle size in group 2 mares during 2<sup>nd</sup> course was 31.4±0.2 mm. mares comes to estrus after short cycling with PGF2α with large follicle of 35 mm can ovulate within 2-5 days Larson (2013). Similarly Urquieta *et al.*, (2009) concluded

that mares administrated with 2500 I.U. i/v ovulates within 24-48 hours. In group 2 mares all mares were covered twice between 12-48 hours post HcG injection at 36 hours intervals. The ovulation status was examined after 24 hours of last covering revealed that



80% (8/10) of mares were successfully ovulated within 36-48 hours of post HcG. The Mean±SEM (P<0.05) of duration of estrus in group 2 mares were 6.3±0.1 days.

Raz *et al.*, (2010) reported that 73% of mares were successfully ovulated after HcG administration and 75% of ovulated mares were conceived. Bucca and Carli (2011) concluded that 90.9 % of mares were successfully ovulated within 24- 48 hours after HcG administration. Morel and Newcombe (2008) reported that mares treated with HcG 1500 I.U. ovulated within 48 hours and pregnancy rates was 65.6 %. In group 2mares the conception rates was 70% (7/10) detected on day 30<sup>th</sup> after last covering.

In tropical conditions during transition period (January- March) there are only few mares show estrus signs with prolonged and irregular estrus. The incidence of large dominant follicles regression without ovulation is very high and conception rates are very low. The application of exogenous hormones to monitor normal estrus length and ovulation is quite effective during transition period. Hormones protocols based on Prostaglandins and HcG will leads to successfully regulates normal length of estrus and ovulation in indigenous mares and high conception rates are achieved.

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