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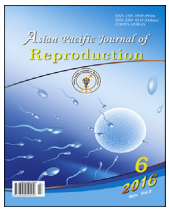

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Factors affecting length of gestation in artificially inseminated Marwari mares of India

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ABSTRACT

Objective: To study the factors affecting length of gestation in foals from artificially inseminated Marwari mares for a period of ten consecutive breeding seasons.

Methods: Gestation length was measured in 126 pregnancies from which viable foals have born. Time of ovulation was confirmed through ovarian ultrasonography in order to determine true gestation length (ovulation to till foaling) as different to previous studies, in which either the mating to foaling interval or from fertilization to till parturition was used for calculating the gestational length. All the mares were inseminated artificially with frozen thawed stallion semen at appropriate time points by monitoring the point near to ovulation. Pregnancy was diagnosed approximately 11–15 d after ovulation initially, and re-affirmed at 30–60 d of pregnancy.

Results: The observed mean gestation length for all the foals was recorded to be 342.20 ± 1.91 (with standard deviation 21.4) d, and ranged from 259 to 388 d. Various factors like parity of the mare, fetal gender, fetal birth weight, season of breeding (Artificial Insemination) and season of foaling were considered for their effect in causing variation on the gestation length in Marwari mares. According to the present study point of view and after compilation of the results, season of breeding and foal birth weight were found to show significant effect in causing true variation of the gestation length in Marwari horses. Other factors like sex of the foal and parity of the mare also had an effect on length of gestation, but whose effect was observed to be non-significant. Over the range of gestation lengths observed, season of foaling was not significantly associated either with gestational length of mare or foal birth weight.

Conclusion: From the current study it can be concluded that the true average gestation length for Marwari foals born is 344.1 ± 0.49 d. The range of gestation lengths was found to be 315–388 d all resulting in viable foals. The length of gestation is not significantly affected by age and parity of the mare. The only factors significantly affecting gestation length are foal gender and month of foaling ($P < 0.05$).

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1. Introduction

Marwari breed of horses are well known for their unique characteristics like majestic look, alertness and resistance to unfavorable conditions. These horses belong to Marwar and Mewar area of Rajasthan, India. The Marwari breed is thought to be resulted as a crossing between indigenous Indian ponies and Arabian horses, having some Mongolian influence. They are famous for their ears that point inward and often touch or overlap and are considered to be the most valued animal genetic resource in the region because of speed, vigor and smartness. This Marwari breed can be distinguished from other indigenous breeds in terms of both physical characteristics (Averages between 152 and 163 cm height) and environmental adaptability. As a consequence of indiscriminate breeding practices and rapid mechanisation, the number of this breed has dwindled and as per livestock census (2011), only a few thousand purebred Marwari exist in India. For preserving the genetic heritage of these Marwari breed populations, it is essential to characterize their reproductive traits and functions.

The knowledge of the length of gestation particularly in mares would make possible for determination and calculation of the date of foaling, which would help in better planning and organization of work in stud farms more efficiently, taking care of a due mare and a foal properly, and facilitates planning for the subsequent breeding season, which is especially important in equine breeding farms that do not use artificial insemination [1] and also in routine equine industry. However, in assuming the near accurate time of foaling makes it extreme difficult in assisting the foaling and it consequently can be harmful and may be problematic for both dam and foal. The inability to predict the accuracy of the time of foaling may incur in extra expenditure on labor and cost of veterinary assistance and also high risk for both dam and foal [2]. Therefore, any factor which may be helpful in predicting the accurate time of foaling would be beneficial to the equine farms and farmers [3].

Pregnancy in equines is known to be long and variable; the time gap between foaling and following pregnancy must be the minimum to shorten the next foaling, in order to achieve optimal yearly interval between foals which will be the aim for every equine owner and stud farm. The mean gestation period in horses varies more when compared to other farm domestic animals like cows, sheep, goat or pigs [4], which is may be due to the fact that their length of gestation period is long and is influenced by both physical and physiological factors that have no influence on the length of gestation of other livestock species like, embryonic dispause in other livestock [5,6]. Several studies have confirmed wide variations in length of gestation to extent in several breed of horses [7,2]. It is assumed that the average gestation length period in mares ranges from 330 to 340 d [4], even though many researchers reported still broader range of the length for normal gestation. Gestational length in the mare has been reported by many authors and researchers previously as 339 [8–10], 334 [11], 344 [1], and 305–382 d [12,13] in Thoroughbred mares. The length of gestation was reported to be ranging from 314 to 363 d [14] in Arabian Horses, 311–358 d in Spanish Purebred Andalusian and 313–357 d for Arabian mares [15]. The length of gestation in Przewalski mares was reported as 326 d [16]. Gestational lengths from 310 to 380 d were reported for viable foals born [17–19] in exotic breeds. Much of the work done on estimation of gestation length in mares is contradictory and conflicting, as it is lacking statistical significance in some cases. A list for duration of gestation lengths of mares for various different breeds as reported by many researchers was reviewed, compiled and presented in the Table 1.

In practice, gestation lengths of mares from 320 to 360 d of duration may be considered to in normal range as acceptable [20,21]. There are many variables of maternal (age, breed, parity, service period, health status) [4], environmental (climate and location, month of breeding, month of foaling, photoperiod) [22,23], parity [21,24], fetal (sex, weight, status of pregnancy, horse/mule fetus) [25,21] and other miscellaneous factors (nutritional status, effect opf male used for breeding, year of breeding, managemental practices like artificial lighting) [26–28] that influence the gestation length [1] in mares (Figure 1). The variability in gestation length is much wider, and this large and wider window of the timing in which live foals can be born indicates that length of gestation in mares is susceptible to both the internal and external factors. Till now, all the studies which have reported about the gestation length in mares of different breeds were about the natural matings, whereas, this would be the first and foremost study which will be reporting about the factors affecting the gestational length in Marwari mares inseminated artificially with frozen thawed semen. Till now all the studies done for estimating gestational length were calculated the time from mating to foaling instead ovulation to parturition [2]. This observation was supposed as equal as the time between ovulation to foaling. However, it is well known and established fact that spermatozoa may survive up to 7 days in the mare's reproductive tract and are still capable of fertilizing the oocyte [29]. With the advent of ultrasonic imaging it is possible to calculate accurate time of ovulation and hence, the precise calculation of length of gestation. The time period from ovulation to foaling might, therefore is better considered the same as fertilization to foaling. To conclude in total, the calculation of gestational length, from fertilization to foaling, has been found to be highly reliable [1] to that of mating to parturition. Taking this fact in to consideration we monitored the ovulation time closely with the help of an ultrasound machine. The objective of the present this study is to determine the mean gestation length in Marwari mares and to study the influence of various selected factors like sex of the foal, season of foaling, parity of a mare, fetal birth weight and breeding conditions on the length of gestation.

2. Materials and methods

2.1. Animals

The animals that were considered for the current study included both the primiparous and multiparous mares aged between 4 and 18 years old. All the animals were managed under uniform managemental and housing conditions. All the animals were fed uniformly and no source of artificial light and no special diet were provided. Mares' were provided with water and feed *ad libitum* at all the time. Farm veterinary and reproductive data records were also used to analyzing and determine date of artificial insemination, time of ovulation, foal birth weight, and foaling dates for each viable foal pregnancy.

2.2. Geographical location of the farm

The present study was carried out in a governmental equine farm which is located in western part of India in the city named Bikaner which falls in the state of Rajasthan. The city, Bikaner is

Table 1
Gestational length observed between different breeds of horses worldwide till now.

	Breed	Gestation length reported	Authors/Reference	Year
1	PoneyBosnien	339.16 ± 0.9	Hrasnika	1944
2	Arabian	333.7	Vesovic	1953
3	Arabian	333.7	Vesovic	1953
4	Throughbred	338.8	Detkens	1953
5	Arabian	343.6 ± 11.4	Pozo-Lora	1954
6	Arabian	343 ± 11.4	Pozo Lora	1954
7	Spanishbred	343.6 ± 12.1	Pozo Lora	1954
8	Spanishbred	343.66 ± 12.1	Pozo-Lora	1954
9	Percheron	342.2	Bettini	1955
10	Belgian	333.8	Bettini	1955
11	Belgian	336.5	Becze	1958
12	Lippiziano	333.5	Ilancic	1958
13	Nonius	328.0	Pavlovic	1960
14	Avelignese	337.86 ± 13.0	Matassino	1962
15	Polish	294–375	Zwolinski	1964
16	Salernitain	340.96 ± 8.7	Salerno and Montemurro	1966
17	Thoroughbred	305–365	Hintz *et al.*	1979
18	Draught horse	343.3	Bos and van der Mey	1980
19	Haflinger pony	341.3	Bos and van der Mey	1980
20	Shetland pony	337.2	Bos and van der Mey	1980
21	Frisian horse	337.7	Bos and van der Mey	1980
22	Arabian	314–361	Demirci	1988
23	Arabian	332.1 ± 3.3	El-Wishy *et al.*	1990
24	Arabian Crossbred; Thoroughbred Crossbred	317–363	Unian and Pereira	1991
25	Thoroughbred	319–364	Hintz *et al.*	1992
26	Quarter Horse	333–343	Pool-Anderson *et al.*	1994
27	Carthusian Spanishbred	322–359	Perez *et al.*	1997
28	Freiberger	307–361	Giger *et al.*	1997
29	Freiberger3donkey	315–369	Giger *et al.*	1997
30	Thoroughbred	315–360	Kurtz Filho *et al.*	1997
31	Carthusian Spanishbred	322–359	Pérez *et al.*	1997
32	Thoroughbred	306–381	Sanchez	1998
33	Standardbred	302–383	Marteniuk *et al.*	1998
34	Spanishbred	322–346	Blesa	1999
35	Thoroughbred	344.1 ± 0.49	Davies Morel	2002
36	Quarter Horse	339–344	Guay *et al.*	2002
37	Thoroughbred	325–339	Allen *et al.*	2004
38	Lipizzaner	334.3 ± 7.3	Heidler *et al.*	2004
39	Carthusian	297–358	Satué	2004
40	Friesian	331.6	Sevinga *et al.*	2004
41	Arabian	340.3 ± 0.63	Valera *et al.*	2006
42	Andalusian	336.8 ± 0.48	Valera *et al.*	2006
43	Criollo	312–364	Winter *et al.*	2007
44	Thoroughbred	322–366	Duggan *et al.*	2008
45	Quarter Horse	317–375	Duggan *et al.*	2008
46	Standardbred	337–340	Villani and Romano	2008
47	Arabian	334.3 ± 0.22	Cilek	2009
48	Heavy draft Mares	334.9 (313–352)	Takahiro Aoki *et al.*	2013

Adapted and modified from Salerno and Montemurro, 1966; Perez et al., 2003 and Sauté et al., 2011.

located in a geographical location of and North Latitude 73°19′ and East Longitude 28°1′ and situated at an altitude of 797 feet above the sea level. It is located in the middle of the Thar Desert and has a hot climate with very low rainfall and high temperatures. During summer temperatures may exceed 45 °C, and in winter they may go down near 4 °C. Environmental factors like altitude, latitude, climate and day length and daily temperatures are found to determine time of foaling [1] partially. Kurtz Filho *et al.* [30] reported that the number of light hours may cause variations in gestation length by influencing the endocrine system of the mare. Keeping this point in view, we intended to study and record the various factors that influence the gestational length of the Marwari mares at this temperate climate.

2.3. Monitoring of the mares for cyclicity and ovulation

All the mares were monitored through Ultrasound machine from the day of onset of estrus symptoms shown with proximity of a stallion and imaging is repeated daily using a linear-array scanner (Kontron SSD-500, France) equipped with a 5.0 MHz, B-mode, linear transrectal probe/transducer. Mares were inseminated when prominent features for ovulation were observed in the preovulatory mature dominant follicle *i.e.*, follicles with 44–45 mm in diameter approximately, and round to spherical shape of the follicle and consistency (hard/soft) of the follicle, and/or uterus (*i.e.*, uterine edema/uterine infection), and examination repeated each 24–48 h until the confirmation of ovulation via ultrasonography. The ovulation time was calculated as the mean

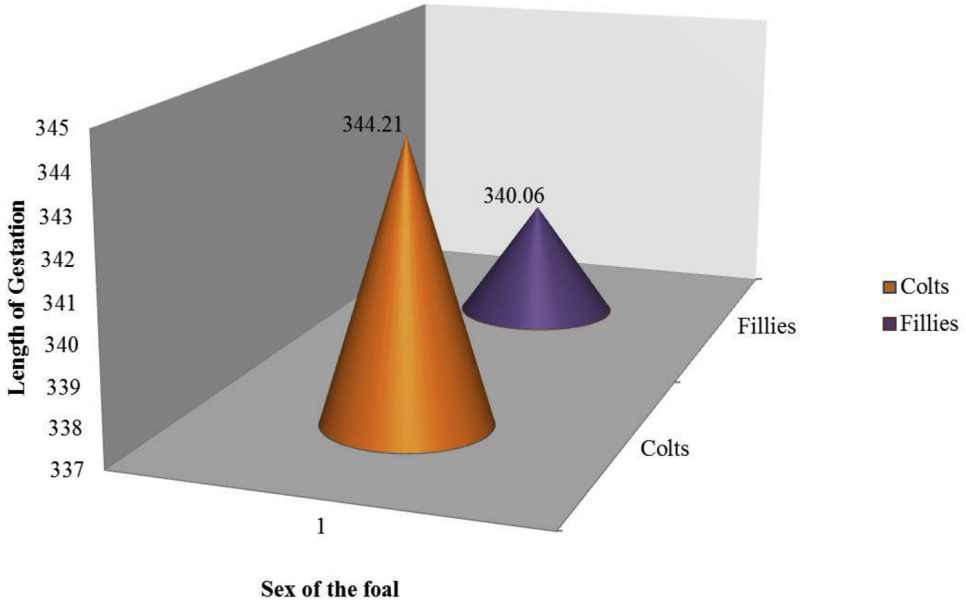

Figure 1. Difference in length of gestation between mares bearing fillies and colts.

of observations when a preovulatory follicle (*i.e.*, anechoic ovarian structure around 40–45 mm in size) was last observed and the first observation of when it was disappeared and corpus haemarrhagicum/corpus albicans was observed. Pregnancy was diagnosed around 11–15 after artificial insemination, by examining the both the uterine horns through ultrasonography, and later reconfirmed on approximately between day 30 and 60. For the present study only gestations with normal parturition and viable newborn foals were considered.

2.4. Cryopreservation of stallion semen and artificial insemination

The semen from Marwari stallions was collected at the Equine Production Campus, National Research Center on Equines, Bikaner, by Artificial Vagina method (Colorado model) using estrus mare as dummy. The stallion semen with progressive motility more than 60% was processed further for cryopreservation using lactose-glucose-EDTA extender. Gel free semen was mixed with Glucose EDTA primary extender [31] in the ratio of 1:1 and centrifuged. The supernatant was discarded and the sperm pellet was dissolved in such a way to get 100–200 million spermatozoa/mL with a modified secondary extender [31]. The diluted semen was kept in the semen cooling cabinet at 4 °C for 2 h as equilibration period. The equilibrated semen was filled in 0.5 mL straws and it was slowly cooled to −100 °C using Biomed planner. Thereafter, the straws were plunged and stored in liquid nitrogen (−196 °C) till use. Thawing of frozen semen was done by immersing the straws in a water bath at 37 °C for 1 min, and 4–5 mL of semen was artificially inseminated in the estrus mare after detecting the suitable period of near ovulation.

2.5. Statistical analysis

To find out the analysis of variance of fixed effects on gestation period, the General Linear Model procedures (SPSS, Version 17.0) were used. The fixed effects considered were season of AI (grouped into three classes, *i.e.* 1 = transitional period, from September to January; 2 = Reproductive season, from February to May; 3 = end of reproductive season, from June to August), Season of foaling (three classes similar to AI class), Sex of the foal (Male-1, Female-2) and birth weight of foal (in three classes *i.e.* 1 = up to 27 kg, 2 $\geq$ 28 to $\leq$34 kg and 3 $\geq$ 35 kg). The statistical model used to analyze growth was:

$$Y_{ijklm} = \mu + A_i + F_j + S_k + B_l + e_{ijklm}$$

Where: Y_{ijklm} = the observation on Gestation length; μ = Overall mean; A_i = Fixed effect of Season of AI (i = 1, 2 & 3) F_j = Fixed effect of season of foaling (j = 1, 2 & 3); S_k = Fixed effect of sex of foal (k = male, female); B_l = Fixed effect of birth weight of foal (l = 1, 2 & 3); e_{ijklm} = effect of random error. Duncan Multiple Range test was used for comparison of significant sub class means by using Post Hoc option.

Subjects were divided into three groups for each AI season and Foaling season (grouped into three class, *i.e.* 1 = transitional period, from September to January; 2 = reproductive season, from February to May; 3 = end of reproductive season, from June to August).

The data were also analyzed for the influence of various factors on gestation length of the mares using a factorial ANOVA model, which included as factors the season of AI, sex of the foal (Male-1, Female-2), bodyweight of the foal (in three class *i.e.* 1 = up to 27 kg, 2 $\geq$ 28 to $\leq$34 kg and 3 $\geq$ 35 kg) and different interactions between the factors. The statistical analysis was performed by GLM procedure of Statistical Package for Social Sciences (SPSS), Version 17.0.

3. Results

3.1. Gestation length in Marwari mares

All the mares from the present study were artificially inseminated with frozen stallion semen at right time points near to ovulation through ultrasonographic monitoring and the mares were diagnosed for pregnancy between 11 and 15 d after ovulation initially, and re-affirmed at 30–60 d after insemination. For the present study a total of 126 gestational lengths have been considered for a period of 10 consecutive breeding seasons. The mean gestation length for all these mares was observed to be

Table 2
Least square means (±SE) of gestation length in mares (Means within a column within an effect with different superscript differ significantly.

	Variable	N	LSM ± SE
Season of AI**	1	40	354.32 ± 5.15[b]
	2	57	340.60 ± 3.94[ab]
	3	29	335.43 ± 4.08[a]
Season of foaling NS	1	32	340.28 ± 4.09
	2	67	350.62 ± 4.60
	3	27	339.46 ± 4.05
Sex NS	Male	65	343.18 ± 2.44
	Female	61	343.72 ± 2.94
Birth weight**	1	26	356.95 ± 3.83[b]
	2	60	339.79 ± 2.66[a]
	3	40	333.63 ± 3.39[a]

a,b and ab denote the statistical difference between the means is significant.
NS = not significant ($P > 0.05$); *$P \leq 0.05$; **$P \leq 0.01$).

342.20 ± 1.91 d with a standard deviation of 21.46 d. The range of length of gestations varied from 259 to 388 d in the current study.

3.2. Effect of season of AI and foal gender on gestation length

The interaction effect of AI season and Sex (Figure 1) [F (2.97) = 3.340, P = 0.04] was found to be significant on gestation length, whose effect size was medium (Partial eta squared = 0.06) [31] (Table 2 and Figure 2). However, simple main effects for interaction of AI season and sex showed no significant interaction. The gestation length was longer for the mares foaling in the months between September and December whereas for the foals with lower body weight (<25 kg), the gestation length tends to be longer (Figure 3). Hence, it is concluded that foaling season and body weight of the foal

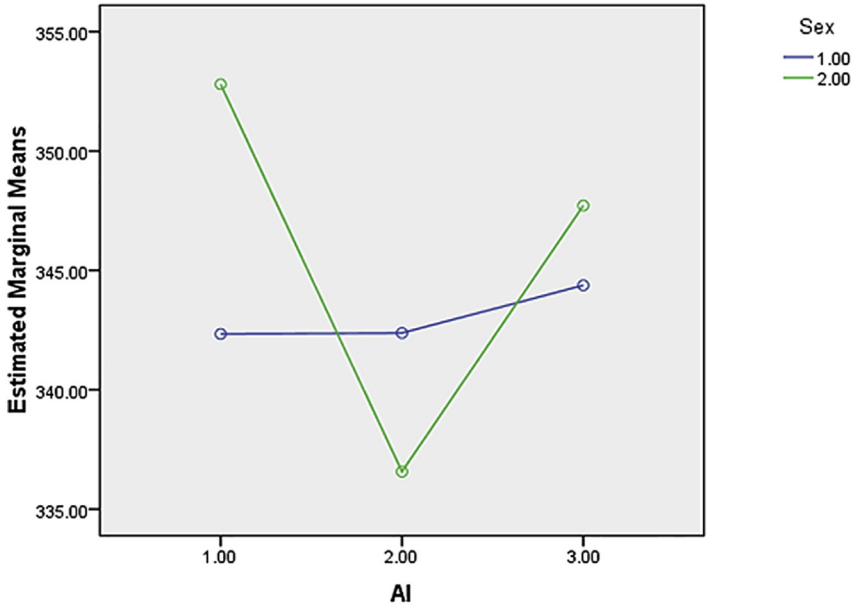

Figure 2. Graphs for interaction of AI season and sex on gestation length.

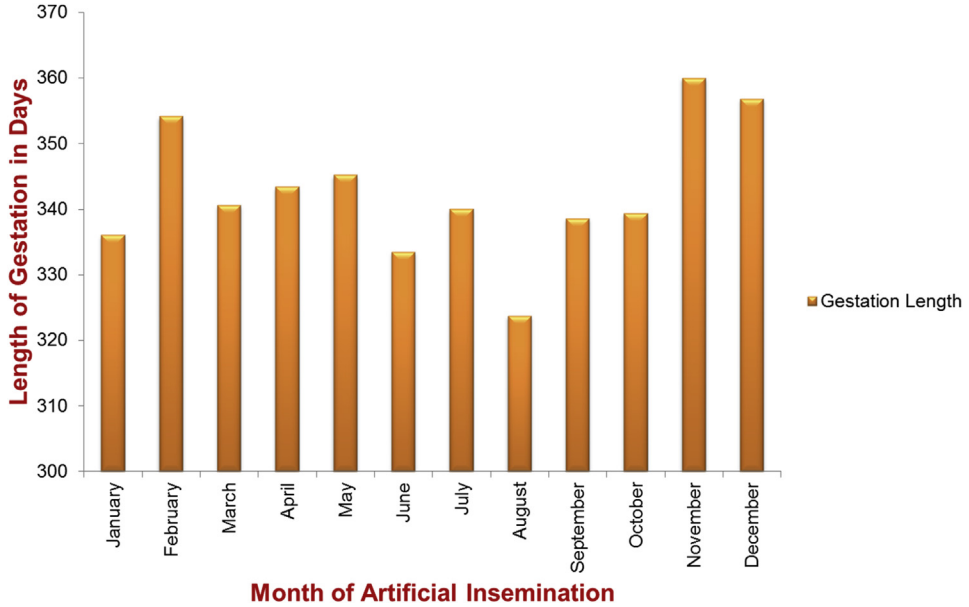

Figure 3. Graph showing the effect of the season of breeding on gestational length.

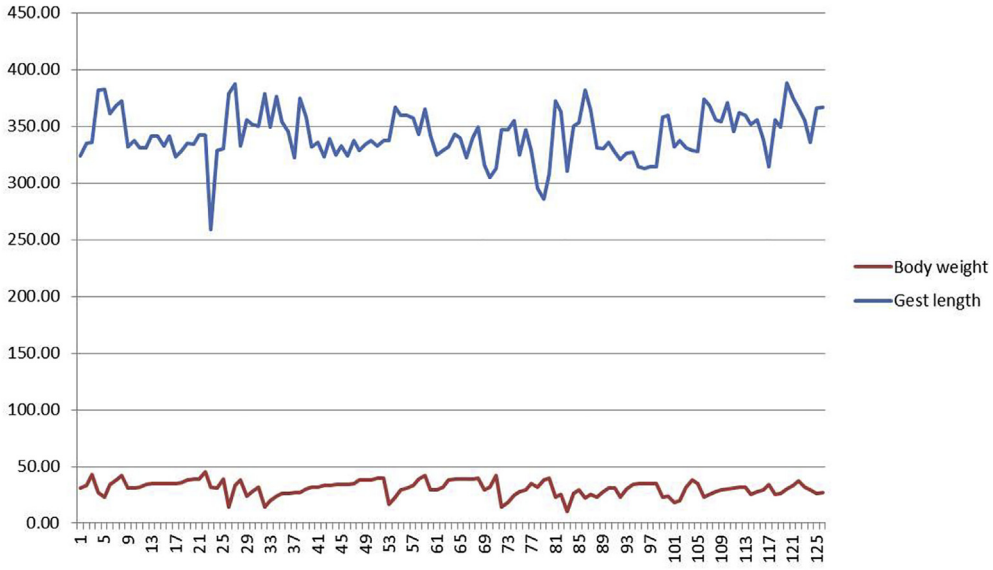

Figure 4. Graph showing the trend between the body weight of the foal born and length of gestation.

significantly affected the gestation length in mares in the present study. Further, the gestation length was longer for the mares foaling in the months between September and December whereas for the foals with lower body weight (<25 kg), the gestation length tends to be longer.

3.3. Effect of parity on gestation length

There was a significant ($P \leq 0.01$) demarcation in length of gestation variation between the primiparous and multiparous mares were noted. We observed that there is an increased length of gestation in primiparous animals compared to multiparous mares, and there is also an increase in the birth weight of the foals born to pluriparous mares than to primiparous mares (*data not shown*).

3.4. Effect of foal birth weight on gestation length

The main effect for fetal body weight [F (2,97) = 7.16, [$P = 0.001$] was significant on length of gestation with the effect of size being large (Partial eta squared = 0.13) [31] (Figure 4). Post-hoc comparisons using the Turkey HSD test indicated that the mean gestation length for Group 1 (<25 kg) (M = 356.14, SE = 3.88) was significantly higher than Group 2 (25–34 kg) (M = 336.94, SE = 2.90) and Group 3 (>35 kg) (M = 336.64, SE = 4.36). However, there was no significant difference between groups 2 and 3 (Table 2).

3.5. Effect of season of foaling on gestation length

Post-hoc comparisons using the Turkey HSD test indicated that the mean gestation length for Group 3 (June–Aug) (M = 352.96, SE = 5.09) was significantly higher than Group 1 (Sept–January) (M = 342.38, SE = 3.21) and Group 2 (Feb–May) (M = 335.46, SE = 2.98) (Figure 4) Groups 1 and 2 did not differ significantly from each other. There was a statistically significant main effect for foaling season [F(2,97) = 4.82, $P = 0.01$]; and the effect size was found to be large (partial eta squared = 0.09) [31] (Table 2 and Figure 5).

Figure 5. Graph showing the difference in gestational lengths for foaling between different months.

4. Discussion

4.1. Gestation length of Marwari mares

The mean gestation length for 126 foals for mares which were inseminated with frozen stallion semen was 342.20 ± 1.91 (SD = 21.46) d, with a range of 259–388 d in the present study. The gestation length for Marwari mares reported in the current study was in agreement with the previous observation of 344 days reported for Northern Hemisphere Thoroughbred mares [1] and Standardbred mares of 343 d [32]. Gestation lengths from 300 to 380 d were reported for various breeds of horses and the present data fits in correlation to the previously reported length of gestations for various breeds as reported and reviewed by Satue *et al.* [2]. The external factors like climate, environment, location and daily variation in the temperature of the location might be the reason for the observed wider variation and range in gestation lengths for mares in the present study.

4.2. Effect of parity of the mare

The increase in the length of gestation in primiparous animal than that of multiparous observed in this study is in agreement with the previous reports [33,34]. It was considered that primiparous mares, which are usually less in age and young mares, have longer gestational lengths due to their unpreparedness both anatomically and physiologically for foaling process. However, Arora, *et al.*, [35] and Sanchez, [36] also recorded the non-significant difference in gestational length between primiparous and multiparous mares. On the contrary Geissler [37] and Flade *et al.* [38] reported gestation lengths increased with age of mares. This may be to due to a decrease in uterine/placental nutritional efficiency [39] and/or the metabolic-hormonal drive to grow [40]. As a consequence of age and multiparous state, these could be of slowing intrauterine growth which prolongs gestation length and delays foaling [39,41]. Age of a mare has a definite effect on the length of gestation for different breeds of horse, the duration of the first gestation shortens progressively to a certain age and then it becomes longer [15].

4.3. Effect of fetal birth weight on length of gestation

In the current study a significant difference in the gestational length was observed between the individual mares bearing heavy and light foals. Mares bearing heavier foals showed less duration of gestation and vice versa. Each species has a genetically programmed development rate and birth process commences in response to a signal, given when the fetus attains an appropriate size and/or maturity [42]. Such a signal could be transuded either by the dam bearing the fetus (*e.g.*, uterine volume), the fetus itself (*e.g.*, nutritional sufficiency) or placenta (*e.g.*, increased fetal for nutrients/single or double). In ruminants, the signal has clearly been shown as glucocorticoid, secreted by the fetal adrenal cortex. In the horse, the fetal cortisol profile increases only in the last 48 h before foaling and maternally administered glucocorticoid does have any effect and does not induce labor, as it does in other domestic animals [42]. In the horse, there is no appropriate evidence supporting the role of the fetus in initiating foaling [30] and it is not clear whether fetal size or maternal stress is important in inducing foaling process. The shorter the length of gestation, and the weight at birth are, the greater the risks for complications [2]. Most of the foals with gestational lengths less than 300 days are observed to be non-viable, due to improper development of fetal organs [43].

4.4. Effect of season of breeding and foaling on gestational length

There was general trend of increasing in length of gestation in mares that were bred in the reproductive season and beginning of the reproductive season to that of that were mated at the end of the breeding season, which is also correlating with the reports earlier reports [10,22,32,35,36,44–48]. Mares inseminated earlier in the season had longer gestation lengths than those bred late in the season, which was in correlation with the Thoroughbred horses from northern hemisphere [1,32] in Standardbred mares and Thoroughbred mares respectively. Environmental temperature and day length and may influence gestation length [1], with shorter length of the day and cooler the ambient temperatures being associated with longer gestation lengths.

4.5. Effect of sex of the fetus on gestation length

There was a difference in the observed gestational length for the fillies and colts born. The length of gestation was found to be increasing in trend in colts to that of fillies born. The difference was observed to be non-significant in their least mean squares.

This observation is also in agreement with the previously reported studies [12,13,21,22,25,28,49]. It is accepted that male-product pregnancies are slightly longer than their female counterparts [1,10,15,22,25,32,44,46–48,50,51]. The reason for the variation of gestational length associated with the gender of the foal has not been completely elucidated yet. However, it is agreed that male body development is greater and faster to females and therefore, the foaling/parturition occurs when the fetal development is complete, then the length of gestation for a colt would be longer [52] It is also hypothesized by some researchers that the difference is due to different endocrine functions of male and female fetuses interacting differently with the endocrine control of foaling [53]. In many species, it is generally accepted that the male fetus has longer gestations than the female fetus; however, the reason of this mechanism still remains un-elucidated [2]. In human pregnancies, this fact has been associated with differences in production of androgen and its action [54] and to sex-chromosome-linked effects [55,56]. Same effect in horses was not yet demonstrated.

From the current study it can be concluded that the true average gestation length for Marwari foals born is 344.1 ± 0.49 d. The range of gestation lengths was found to be 315–388 d all resulting in viable foals. Age and Parity of the mare did not show any significant effect on gestation length. The factors significantly affecting gestation length were foal gender and month of foaling ($P < 0.05$). The significant effect of foal birth weight and season of breeding or mating on gestation length is unclear but is likely, at least in part, to be due to variation in the climate and the environment of the location of the study place, nutritional uptake between individual mares during gestation. However, the results are significant in themselves, with the use of ultrasonic scanning, as the present study

gave an accurate indication of gestation length to date in Marwari mares.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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