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Original Article

A survey on relationship between body condition score, vaginal pH and some serum biochemical parameters and sex ratio of their offspring in Arabian mares

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Abstract

Among various animal species, fetal sex determination and the use of methods to make earlier diagnosis of fetus sex possible are of great interest. There are several assumptions to predict the sex ratio of offspring from a given parent, including the relationship between maternal body condition and some factors in the environment. The present study conducted on 25 Arab mares in Ahvaz, Khuzestan, in the southeast of Iran, to investigate the relationship between maternal body condition score (BCS), vaginal pH, blood glucose, triglyceride, cholesterol and urea levels in mares and offspring sex ratio, one month before mating and at mating time, and also the above-mentioned parameters' changes during these periods. The results showed that the maternal BCS had no significant effect on predicting offspring sex one month before mating, while its effect on offspring sex was significant at mating time. As was expected, with one-unit increase in maternal BCS one month before mating, the proportion of males to females offspring increased by 4.81 times. The U-Mann-Whitney statistical test showed that the mean score of body condition in mares, who had a colt, was significantly better (higher) than in mares with fillies at each time point of the study. However, no significant relationship was found regarding changes in body condition score and offspring gender. It was also found that other variables had no significant effect on offspring sex at any time of evaluation. In general, it can be claimed that BCS assessment can play an effective role in predicting offspring sex ratio.

Key word: Arabian horse, BCS, Biochemical parameters, Sex ratio, Offspring

Introduction

Fetus sex determination and the use of a method that can detect the fetus sex as quickly as possible is very favored among different animal species and even humans. Regarding the animal species raised on farms, there is a preference for a particular gender, which can be due to commercial implications. (Aurich and Schneider 2014). There are several assumptions to predict the sex ratio of offspring from a given parent; for instance, the relationship between maternal body condition at mating time and also its changes with offspring sex. (Cameron et al. 1999, Cameron and

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Linklater 2000). Based on the Trivers &Willard theory, there is a belief stating that the sex ratio of offspring in mothers with good body condition will be more towards the male. However, if the body condition of these mothers is not ideal, female births will be more expected. (Cameron and Linklater 2007, Douhard 2017). Some researchers believe that changes in BCS could have further predictive value than BCS evaluation, only at a given time. (Cameron and Linklater 2007). For example, a study by Clutton and Iason (1986) showed that among mares with more favorable body condition at insemination time, the number of male offspring were significantly higher. On the other hand, according to some studies, the external environment also signals to the fetus through the mother's uterine and affects it (Allen et al. 2015, Aurich and Schneider 2014). For example, Aurich and Schneider (2014) held that in many species a male fetus will have a higher chance of survival in a glucose-rich uterine environment than a female embryo. (Aurich and Schneider 2014). Researchers suggested that the survival power of male and female embryos differs in response to the presence of glucose as well as survival in environments with different concentrations of glucose (Gutierrez et al. 2001). Given the above, the present study aimed to investigate the relationship between some factors, such as maternal BCS, vaginal pH, level of glucose, triglyceride, cholesterol, and urea in blood of mares and the offspring sex ratio, one month before mating and at mating time and its changes during these periods of time.

Materials and Methods

This study was carried out on 25 Arabian mares aged 5 to 22 years kept in the equestrian club in Ahvaz. Samples were obtained one month before mating and at mating time. Questionnaire of mares' characteristics, including age, number of births and diet were recorded. After ensuring that the horses are healthy by clinical examination and obtaining a history from the owner, sampling from the jugular vein was performed. Blood samples were then transported to the clinical pathology laboratory under standard conditions, in test tubes containing clot activator. After 1 to 2 hours, when the samples' temperature reached ambient temperature, the blood clot against the tube wall was removed by a plastic applicator, and the tubes were centrifuged at 3000 rpm for 15 minutes. The produced serum was finally kept at -20 ° C until biochemical tests. Serum biochemical parameters such as glucose, cholesterol, triglyceride and urea were evaluated using Pars Azmoun kits, with the help of the autoanalyzer on the basis of spectrophotometry.

Maternal BCS, on the other hand, was determined by two individuals using the Henneke system (Henneke et al. 1984). The pH test paper strip was further used to assess vaginal pH of mares. To do so, first, a strip of paper was applied to the mare vagina for about 2 minutes and finally the color change was compared with the guide color spectrum to determine pH.

The data were analyzed by using logistic regression model, independent t-test, and the U Mann-Whitney test in SPSS software version 22.

Results

In the present study, out of the total 25 mares studied, 16 colts and 9 fillies were born.

Body Condition Score

Results of the present study revealed that the mares' BCS, one month before mating had no significant effect on offspring sex ratio (Table 1), whereas the mares' BCS at mating time was associated significantly with offspring sex ratio. It was also predicted that the odds ratio of males to females will increase by 4.81 times per unit with regard to the increase in mares' BCS. ($P<0.05$). (Table 2). Furthermore, use of logistic regression test to assess the impact of changes in mares' BCS, within one-month interval, did not show any significant effect on offspring sex. (Table 3). It is further worth mentioning that the U Mann-Whitney test of maternal BCS in both groups with male and female offspring, one month before mating and at mating time, showed that the average score of BCS of mothers with male offspring was significantly better (higher) at any given time than the mares with female offspring. But, the above test showed no significant relationship between changes in maternal BCS and sex ratio (Table 4).

Table 1. Influence of Body Condition Score on the offspring sex ratio. (A month before mating)

Variable	Coefficient	SE Coefficient	Z	P	Odds Ratio
Constant	-8.04094	4.52401	-1.78	0.076	-
BCS	1.57154	0.838289	1.87	0.061	10.5

Table 2. Influence of Body Condition Score on the offspring sex ratio. (At mating time)

Variable	Coefficient	SE Coefficient	Z	P	Odds Ratio
Constant	-12.8838	6.05362	-2.13	0.033	-
BCS	2.35163	1.06026	2/22	0.027	4.81

Table 3. Influence of Body Condition Score change on the offspring sex ratio.

Variable	Coefficient	SE Coefficient	Z	P	Odds Ratio
Constant	0.485056	0.439707	1.1	0.27	-
BCS	0.498354	0.796559	0.63	532.0	1.65

Table 4. Mean± SD of relationship between Body Condition Score a month before mating and at mating time and the offspring sex ratio

Offspring Sex	BCS a months before mating	BCS at mating	BCS Change
Mare	5.81 ± 0.72	6 ± 0.57	0.18 ± 0.44
Female	5.22 ± 0.5	5.44 ± 0.63	0.22 ± 0.5
p Value	0.042	0.036	0.86

Vaginal pH and serum biochemical parameters

Results of logistic regression test related to other evaluated variables of mares, including vaginal pH, serum glucose, cholesterol, and triglyceride as well as serum urea indicated that these items had no significant effect on offspring sex ratio, a month before mating (Table 5), and at mating time (Table 6). Moreover, changes of these variables did not have any significant effect on offspring sex ratio (Table 7). Also, unpaired T-test for each of the above mentioned variables, a month before mating and at the time of mating (Table 8), and their changes in these periods of time (Table 9) did not have any significant differences between the two groups.

Table 5. Influence of vaginal pH, serum glucose, cholesterol, triglyceride and urea level on the offspring sex ratio (A month before mating)

Variable	Coefficient	SE Coefficient	Z	P	Odds Ratio
Constant	0.722873	6.81812	0.11	0.916	-
Vaginal pH	-0.467019	1.20326	-0.39	0.689	0.63
Glucose	0.0505375	0.0392372	1.29	0.198	1.05
Cholesterol	-0.0004856	0.0273523	-0.02	0.986	1
Urea	-0.0443582	0.0528201	-0.84	0.401	0.96
Triglyceride	0.0029373	0.0509542	0.06	0.954	1

Table 6. Influence of vaginal pH, serum glucose, cholesterol, triglyceride and urea level on the offspring sex ratio (At mating time)

Variable	Coefficient	SE Coefficient	Z	P	Odds Ratio
Constant	0.722873	6.81812	0.11	0.916	-
Vaginal pH	-0.467019	1.20326	-0.39	0.689	0.63
Glucose	0.0505375	0.0392372	1.29	0.198	1.05
Cholesterol	-0.0004856	0.0273523	-0.02	0.986	1
Urea	-0.0443582	0.0528201	-0.84	0.401	0.96
Triglyceride	0.0029373	0.0509542	0.06	0.954	1

Table 7. Influence of vaginal pH, serum glucose, cholesterol, triglyceride and urea level change on the offspring sex ratio

Variable	Coefficient	SE Coefficient	Z	P	Odds Ratio
Constant	0.720408	0.483582	1.49	0.136	-
Vaginal pH	-0.103716	1.11289	-0.09	0.926	0.9
Glucose	-0.0298546	0.0238872	-1.25	0.211	0.97
Cholesterol	0.0101804	0.0477257	0.21	0.831	1.01
Urea	0.0243056	0.0429386	0.57	0.571	1.02
Triglyceride	0.0066319	0.00640544	0.1	0.918	1.01

Table 8. Mean± SD of relationship between vaginal pH, serum Glucose, Cholesterol, Triglyceride and Urea level (A month before mating and at mating time) and the offspring sex ratio

Foals Sex	Vaginal pH A	Glucose (mg/dl) A	Cholesterol (mg/dl) A	Urea (mg/dl) A	Triglyceride (mg/dl) A	Vaginal pH B	Glucose (mg/dl) B	Cholesterol (mg/dl) B	urea (mg/dl) B	Triglyceride (mg/dl) B
Male	6.53± 0.59	90.5± 17.98	85.43± 18.62	30.68± 9.51	16.31± 7.31	6.4± 0.61	88.12± 16.2	85.75± 15.57	25.81± 5.76	16.56± 8.36
Female	6.38± 0.48	80.22± 16.99	85.11± 10.71	33.66± 7.17	16.66± 11.91	6.33± 0.43	89.22± 14.75	87.77± 13.77	29.11± 9.86	16.66± 6.55
P Value	0.545	0.175	0.962	0.423	0.927	0.756	0.868	0.878	0.823	0.975

A: A month before mating; B: At mating time

Table 9. Mean± SD of relationship between changes in vaginal pH, serum level of glucose, cholesterol, triglyceride and urea (the interval between a month before mating and at mating time) and the offspring sex ratio

Foals Sex	Vaginal pH change	Blood glucose change	Blood cholesterol change	Blood urea change	Blood triglyceride change
Male	-0.12 ± 0/46	-2.37 ± 16.02	0.31 ± 11.36	-0.87 ± 11.4	0.25 ± 6.87
Female	-0.05 ± 0/46	9 ± 24.09	-0.33 ± 7.92	-4.55 ± 10.1	0 ± 8.74
P Value	0.723	0.169	0.882	0.429	0.938

Discussion

The present study was carried out on 25 Arabian mares to examine some maternal traits and their relationship with the sex of the offspring.

There are several hypotheses to predict offspring sex ratio from a given parent. Maternal BCS have attracted researchers' attention, among other hypotheses. According to Trivers and Willard, this idea suggests that mares with good condition invest more in colt, whereas poor-condition mares invest more in fillies (Douhard 2017, Cameron and Linklater 2007, Cameron 2004). In fact, this theory holds that in order to predict sex, attention must be focused more on the status of the mother (Cameron 2004). Few definitive studies are available rejecting or confirming this theory in mammals which, in turn, makes their interpretation difficult (Cameron and Linklater 2007). After reviewing more than 400 studies on different species of mammals, Cameron and Linklater (2007) stated that only 33% of the studies are consistent with the Trivers & Willard (T&W) hypothesis, and in most studies, there is no consistent relationship between maternal BCS and offspring sex ratio. Besides, very few of these studies have rejected the T&W theory (Silk and Strum 2010). Recent studies have shown that differences in the results of studies are probably due to differences related to the timing of BCS calculation (Sheldon and west2004). For instance, some researchers believe that changes in BCS could have further predictive value than BCS evaluation, only at a given time. (Cameron and Linklater 2007). A study by Clutton and Iason (1986) showed that among mares with more favorable body condition at insemination time, the number of male offspring were significantly higher.In another study, Cameron et al. (1999) evaluated the effect of mares' BCS, around the fertilization time, on the offspring sex ratio of 400 Caimanava horses during a breeding period when 70% were fertilized; this work indicated that better BCS near fertilization was significantly associated with increase in male offspring proportion and vice versa. They also claimed that examining the maternal body condition and its relationship with the sex of offspring around pregnancy is more predictive than that in pregnancy. A study by Cameron and Linklater (2007), on horses in New Zealand proved that changes in maternal BCS, at a time close to insemination, significantly divert offspring sex ratio so that 80% of mares with positive changes in nutritional and physical status hadcolts; However, they stated that the role of changes in maternal BCS at times closer to inoculation was more predictive.

Predicting the offspring sex ratio on the basis of mother's body condition has also been common among red deer, roe deer, adult ewes, barbaric sheep, domestic pigs and a number of other species. (Rosenfeld and Roberts 2004). For example, Roche et al. (2006) verified that the number of male calves born to mothers, who did not lose their BCS between calving and conception were 1.8 times more than those who lost one unit of body condition. A study on red deer, which conducted over a 28-day period prior to mating (Luna-Estrada et al. 2006) stated that in mothers who had weight gain during this period, the sex ratio of offspring was significantly higher in favour of males. Contrary to the above results, Polak et al. (2015) found conflicting findings in a study on 11644 female goats; they believed that their study did not show any significant relationship between maternal BCS and offspring sex ratio.

In general, it can be concluded that the results of BCS assessment in the present study were in line with some studies. Perhaps the reason for the insignificant relationship between the changes in BCS and offspring sex ratio could be explained by the small amount of these changes in the sense that the average rate of the change has not reached one unit.

Glucose is another important factor in fetal sex determination that some researchers have considered. For instance, Cameron (2004) and Gutierrez et al. (2001) stated that changes in blood glucose levels due to maternal BCS changes is an important factor in determining fetal sex. According to some researchers, external factors transmit signals to the fetus through mother's uterus and affect it. (Allen et al. 2015, Aurich and Schneider 2014). For instance, it is stated that in many species male fetus has a higher chance of survival in the uterus environment enriched with glucose than the female fetus (Aurich and Schneider 2014). Furthermore, two studies by Kimura et al. (2005) and Cameron et al. (2008) carried out on cows and rats, respectively, revealed that increased glucose levels in uterus after pregnancy are associated with an increase in male births. Other studies have also suggested that the survival power of male and female embryos differs in response to the presence of glucose as well as survival in environments with different concentrations of glucose (Gutierrez et al. 2001). On the other hand, it has been reported that high concentrations of glucose in uterus (similar to serum concentrations) during the early stages of embryo division could be destructive in some species. For example, in cows, it has been declared that increasing uterine glucose level up to 5.6 mmol (similar to the serum concentration) could be harmful to the cow's embryo so that it stops a large number of female morulas from becoming blastocysts (Peippo et al. 2001), as a result, the sex ratio is increased under such conditions and more male fetus is produced. Gutierrez et al. (2001) showed that in the absence of glucose in embryo culture medium, the ratio of male to female was equal, and by adding glucose to the medium, the proportion of male blastocysts increased significantly. Machado et al. (2001) further asserted that in diabetic rats, male birth would be significantly higher due to high blood glucose levels and therefore higher glucose levels in the uterus. Another study by Kimura et al. (2005) verified that increasing glucose levels exceeding 2.5 mmol in embryonic medium significantly increased male offspring. Another study by Bermejo-Alvarez et al. (2012) confirmed that male blastocysts grew more in glucose-rich embryo culture medium, but no significant difference was observed in offspring sex ratio.

In short, in most of the studies above, the role of glucose in modifying the sex ratio of offspring was mentioned, in the way that increased glucose levels led to increased male sex ratio in offspring. In this study, however, assessment of blood glucose level at each time point and its changes over the aforementioned periods did not display a significant relationship with offspring sex ratio. Also, results showed that blood glucose did not affect sex determination of foal ($P<0.05$). It should be emphasized that most of the above studies were completed *in vitro*, and most of them were conducted on mice embryos and through altering the embryo culture medium. Only in Machado's (2001) study on mice, blood glucose level was considered and since the mice were diabetic, their blood glucose level was higher than normal. Therefore, it might be argued that, in this study, the ineffective role of glucose in determining offspring sex ratio could be due to the fact that blood glucose was within the normal range in all the mares, whereas higher concentrations of glucose is likely to be required to produce desired effects.

Triglyceride and cholesterol are other blood parameters examined in this study. Although several studies have focused on the role of fat in animals' diet and its effect on offspring sex ratio, no measurement of blood triglyceride and cholesterol levels and their relationship with offspring sex ratio has been reported in any studies. Another study by Alexenko et al. (2007) on rats showed that in rats who received high saturated fat diet, the sex ratio was higher towards male compared to the control group and those fed on a diet containing a controlled amount of fatty acid. In addition, a study conducted on sheep reported that in ewes receiving diets enriched with unsaturated fatty acids, the sex ratio tended towards male births (Green et al. 2008). In the same way, in relation to humans, Mathews and Hamilton (2005) obtained similar results to those of Green et al. (2008). Some researchers have claimed that in female rats, a high-fat diet leads to higher levels of estradiol, and factors, such as serum steroids and free fatty acids are significantly associated with dietary fat levels (Whyte et al. 2007). On the other hand, Zhang et al. (2007) reported that mice exposed to higher levels of estradiol had significantly higher sexes ratio towards male than controls.It has been found that high testosterone levels produce more boys in humans as well. Austad and Sunquist (1986) also noted that in opossums and humans, an increase in dietary free fatty acids, and especially unsaturated fatty acids, would lead to more male births. Results of the present study are likely not to be significant due to the high similarity of the diets received by the mares under study and also due to the serum levels of the variables to be in the normal range.

There was no significant relationship between blood urea and sex of offspring ($P<0.05$). This may be due to the fact that all specimens were within the serum normal range and also the similarity of all mares' diet. No similar study was found in the related literature.

Vaginal pH has a remarkable effect on sperm activity before and during fertilization. Sperm motility and its survival, sperm capacity and its acrosomal reaction are highly affected by environmental pH. For example, one study showed that millions of sperm die during mating because they are exposed to low pH (Goodall and Roberts 1976). Some researchers further believed that vaginal pH is one of the factors that can affect fetal sex. For instance, a study by Pratt et al. (1987) on golden hamsters claimed that an abnormal increase or decrease in vaginal pH could interfere with the sex ratio of offspring. But few studies are available about this effect. Moreover, Minkoff et al. (1985) reported that the rate of female births was higher in women with *Trichomonas vaginalis*, and it was attributed to the increased pH of the environment by these protozoa. Oyeyipo et al. (2017) also announced that the resistance of sperms containing X and Y chromosomes is not the same under different environmental conditionsin the way that sperm with Y chromosome in alkaline condition and sperm with X chromosome in acidic condition are more stable. They, additionally, claimed that the sperm containing Y chromosome is more motile under normal conditions; such sperm will lose its stability by being exposed to acidic pH and oxidative stress. The reason could be attributed to the larger cell space in the sperm containing X chromosome, and therefore the higher the amount of regulatory proteins and buffering phosphates. (Cui and Matthews,1993). On the other hand, a study by Balaci et al. (2009) conducted on mice in which one group was exposed to daily vaginal washing with acidic substances and the other group with alkaline substances reported that vaginal pH had no significant effect on offspring sex.

Probably the reason of the insignificance results for this study is due to the small number of studied population and with the increase of the statistical population, a different result may be achieved.

Conflict of interest

The authors declare there is no conflict of interest.

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