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Characterization of the Development of Foals in Natural Mating and Embryo Transfer

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ABSTRACT

***Background*:** Several reproductive biotechnologies are used in horses and embryo transfer (ET) is one of the most frequent in countries like Brazil and the USA. It has been shown that animals originated by new reproductive technology can have compromised growth in extreme conditions, however there are still only a few researches on the influence of embryo transfer over lactating foals under different raising methods. Due to the lack of studies on ET products development and the need to understand the process and it's relate with lactating foal's growth, the aim of this study was to evaluate the influence of the embryo transfer and natural mate over the newborn foals.

***Materials, Methods & Results*:** From a group of 20 mares, 20 Quarter Horse lactating foals were used (10 natural matting + 10 embryo transfer). The mares weren't given any concentrate feed during whole gestation and lactation. Foals were kept with the mare, however was used creep feeding to give commercial feed *ad libitum*. The foals biometric evaluation were made at the day of birth (day 0) and at 30^{th}, 60^{th}, 90^{th}, 120^{th} and 150^{th} day (weaning) for the following parameters: body mass (BM), height at the withers (H), heart girth (HG), cannon bone girth (CBG) and fat mass percentage (FMP). The fat thickness percentage at rump it was determined by ultrasound device. All statistics were considered significant when a $P < 0.05$ was detected. It wasn't observed corporal mass differences between the groups of mares and the group of stallions. Milk's components evaluation hasn't showed differences for the assessed parameters on both mare groups. Evaluating lactating foals' development, significant differences were observed for height (H), heart (HG) and cannon bone girth (CBG) and body mass (BM).

***Discussion*:** In this actual research, Natural Mating (NM) and Embryo Transfer mares exhibited similar corporal mass ($P > 0.05$) and so did foals at birth ($P > 0.05$). However, by the time of weaning, body mass (BM) was 25% higher on ET foals group. These results indicate some influence of ET on foals' growth since milk's composition on both groups (G-NM and G-ET) was similar ($P > 0.05$), as well as dietary and sanitary handling that foals and mares were submitted to. It is a fact that one's phenotype is determined not only by its genotype, there is evidence that environmental modification during intra uterine period, as well as extra uterine environment may affect epigenetic features. Changes of physical and skeleton progress patterns on suckling foals remained after weaning. However it could be observed that the present study foals had their development under proposed parameters according to prior studies, which affirm that animals have to duplicate their birth weight at the first month of age and should be five times higher at weaning. It stands out that ET treatment foals reported slightly high values indicating this research and future studies relevance to assure if growth beyond observed commonly is beneficial or damaging. In conclusion, there is little influence of the reproductive method over the lactating foal's development when we compered two groups of foals obtained from natural mate and embryo transfer. However, foals from embryo transfer had large body mass at weaning when compared with natural mate group. Finally, the mares' milk composition was similar between both groups of mares used in this study.

Keywords: biometry, equine, milk, postnatal, reproductive biotechnology.

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INTRODUCTION

Different researcherstried to demonstrate foal's first months of development, under several handling methods and reproductive systems. It is known that foal's growth is directly related to genetic potential, nutrition, exercise and hormonal balance, and these variables are strongly determined by the intra or extra uterine environment. It has been shown that animals originated by new reproductive technology can have compromised growth in extreme conditions [1]. Inside the uterus, fetus growth can be affected by the mare's health and nutrition, hormones and even the uterus capacity of keeping gestation, these maternal influences have been shown in different species [16].

Several reproductive biotechnologies are used in horses and embryo transfer (ET) is one of the most frequent in countries like Brazil and the USA. This technique turns genetic enhancement faster and improves reproductive efficiency, increasing the offspring number in athletic mares or even those, which have some degree of infertility [22]. Moreover, the mare size is expected to influence milk's quality and quantity [3] and this act is important aspect of the newborn foals.

There is little information about the effects of embryos transfer techniques over the fetal and newborn foals, principally in large-scale programs. Due to the lack of studies on embryo transfer newborn foals development and to understand some of this aspect of the foal's development, we developed a research that evaluated the influence of the embryo transfer and natural mate over the newborn foals.

MATERIALS AND METHODS

Animals and management program

For this experiment, 20 Quarter Horse lactating foals were used, from a group of 20 mares. These mares were divided in two groups; one with 10 Quarter Horses mares and that used a natural mate (natural mate group) and another group with 10 recipient mares from various breeds (embryo transfer group). All of the mares were multiparous, healthy and had no reproduction problems history.

All animals were in Lagoa do Carro-PE, Brazil (07°5'1"S 35°19'11"W). This horse farm had pasture with *Panicum maximum cv. Massai* and *Cynodon dactylon cv. Tifton* 68, and all animal had free access to water and equine mineralized salt. The mares weren't given any concentrate feed during whole gestation and lactation. Foals were kept with the mare, however was used creep feeding to give commercial feed *ad libitum* (Guabi® Equitage Potro E[1]; 19% Crude Protein, 5% Ether Extract and 14,44MJ/kg Digestible Energy) during all lactation period.

Foals biometric measurements

The foals biometric measurements were made at the day of birth (day 0) and at 30th, 60th, 90th, 120th and 150th day (weaning) for the following parameters: corporal mass (CM), height at the withers (H), heart girth (HG), cannon bone girth (CBG) and fat mass percentage (FMP). The fat thickness percentage at rump it was determined by ultrasound device Shenzhen Mindray DP2200Vet[2] (5MHz) by measuring rump fat thickness [9,25]. The mares were weighed soon after foaling and stallions at the mating day.

Mares'milk collection and analysis

Mares' milk was obtained manually without use of oxytocin, one hour after foaling and offspring segregation therefore helping udder's milk accumulation. Samples were taken from both teats and then transferred to a recipient containing Bronopol. It was cooled and taken to PROGENE laboratory, at UFRPE Animal Science Department, analyzed by Bentley Combi 2300[3] device and determined fat, protein, lactose and total solids percentages.

Statistical Analysis

Statistical analysis was performed using the program ANOVA. The variables were analyzed using Holm-Sidak's test. It was used the Sigmaplot 13.0 program. All statistics were considered significant when a $P < 0.05$ was detected and results were expressed in mean +/- standard error.

RESULTS

Evaluating lactating foals' development, significant differences were observed for height (H), heart (HG) and cannon bone girth (CBG) and corporal mass (CM) ($P < 0.05$), but the contrary occurred to fat mass percentage (FMP) ($P > 0.05$) [Table 1]. Embryo transfer group had higher H, HG, CBG and CM than NM group when they were 4 months old ($P < 0.05$) but, at weaning, the difference was not present ($P > 0.05$). However CBG and BM had difference between groups ($P < 0.05$).

Indeed, it wasn't observed corporal mass differences between the groups of mares ($P > 0.05$) and the group of stallions ($P > 0.05$). Milk's components evaluation haven´t showed differences for the assessed parameters on both mare groups ($P > 0.05$) [Table 2], but the colostrum presented different protein (G-NM= ~4.7%; G-ET= 6.9%) and lactose (G-NM: ~5.0%; G-ET= ~4.0%). Also, the results showed a significant difference between mares and stallions corporal masses ($P < 0.05$) [Table 3], males were slightly heavier than females.

Table 1. Quarter Horse lactating foals' biometric indexes born from natural mating (n = 10) or embryo transfer (n = 10) from birth to weaning.

Biometric Evaluation	Fase of development					
	At Birth	1st Month	2nd Month	3rd Month	4th Month	5th Month
			Wither Height, cm			
NM	87.65 ± 1.71	97.25 ± 1.17	103.00 ± 4.11	107.80 ± 3.01	112.10 ± 3.78	115.90 ± 2.92
ET	88.65 ± 1.23	98.40 ± 0.89	105.00 ± 3.86	111.10 ± 4.15	117.20 ± 3.05*	119.30 ± 2.98
			Heart Girth, cm			
NM	76.15 ± 1.25	95.60 ± 1.26	104.80 ± 4.98	112.20 ± 5.83	120.90 ± 5.38	123.60 ± 4.40
ET	77.00 ± 1.52	100.10 ± 5.24	110.55 ± 4.85	117.85 ± 5.10	127.50 ± 5.42*	129.70 ± 4.30
			Cannon Bone Girth, cm			
NM	12.35 ± 0.25	13.25 ± 0.21	13.80 ± 0.13	14.30 ± 0.11	15.05 ± 0.14	15.35 ± 0.20
ET	12.80 ± 0.19	13.75 ± 0.20	14.25 ± 0.11	15.15 ± 0.15*	15.90 ± 0.10*	16.55 ± 0.14*
			Body Mass, kg			
NM	40.10 ± 1.74	78.80 ± 2.12	100.60 ± 3.88	119.40 ± 5.83	148.20 ± 5.31	155.70 ± 5.50
ET	42.40 ± 2.33	92.80 ± 4.43	125.20 ± 4.52*	147.70 ± 5.77*	170.40 ± 6.58*	179.90 ± 6.01*
			Fat Mass Percentage, %			
NM	9.31 ± 0.14	9.37 ± 0.14	9.42 ± 0.09	9.48 ± 0.08	9.53 ± 0.04	9.51 ± 0.09
ET	9.18 ± 0.06	9.25 ± 0.03	9.42 ± 0.09	9.48 ± 0.12	9.52 ± 0.13	9.53 ± 0.10

*Presence means $P < 0.05$ by Holm Sidak's test when comparing treatments from the same development phase for the same parameter. (NM= Natural Mating; ET= Embryo Transfer).

Table 2. Colostrum and milk's composition from mares kept in pasture, expressed as a dry matter percentage.

Compound	Lactation Period					
	Delivery	1st Month	2nd Month	3rd Month	4th Month	5th Month
			Fat, %			
NM	1.91 ± 0.24	1.75 ± 0.27	1.17 ± 0.10	0.89 ± 0.18	0.96 ± 0.11	1.35 ± 0.46
ET	1.22 ± 0.30	1.18 ± 0.15	1.18 ± 0.09	1.06 ± 0.47	1.27 ± 0.09	0.79 ± 0.22
			Protein, %			
NM	4.71 ± 0.63	2.21 ± 0.04	1.91 ± 0.11	1.79 ± 0.08	1.54 ± 0.07	2.15 ± 0.44
ET	6.87 ± 1.22*	1.81 ± 0.06	1.85 ± 0.10	1.64 ± 0.13	1.46 ± 0.07	1.59 ± 0.03
			Lactose, %			
NM	4.98 ± 0.21	6.28 ± 0.05	6.52 ± 0.10	6.64 ± 0.07	6.57 ± 0.06	6.63 ± 0.03
ET	3.95 ± 0.44*	6.55 ± 0.07	6.47 ± 0.09	6.71 ± 0.07	6.64 ± 0.05	6.63 ± 0.03
			Total Solids, %			
NM	12.58 ± 0.44	11.55 ± 0.25	10.92 ± 0.13	10.47 ± 0.23	10.22 ± 0.19	10.66 ± 0.69
ET	12.68 ± 0.65	10.86 ± 0.12	9.89 ± 0.11	10.80 ± 0.52	10.86 ± 0.10	10.21 ± 0.24

*Presence means $P < 0.05$ by Holm Sidak's test, when comparing same lactation period. (NM= Natural Mating; ET= Embryo Transfer).

Table 3. Mares' (n = 20) and stallions (n = 5) mean corporal mass.

Group	Corporal Mass (kg)
Mares (NM)	471 ± 27^{B}
Mares (ET)	444 ± 36^{B}
Stallions (NM)	546 ± 16^{A}
Stallions (ET)	544 ± 16^{A}

Means in the same column followed by different letters indicate $P < 0.05$ at Holm-Sidak's test. (NM= Natural Mating; ET= Embryo Transfer).

DISCUSSION

Different studies provide evidence that recipient mare and expected embryo sizes may interfere on foal's development from birth to adult age [1,2,15]. In this actual research, NM and ET mares exhibited similar corporal mass ($P > 0.05$) and so did foals at birth ($P > 0.05$). However, by the time of weaning, body mass (BM) was 25% higher on ET foals group. These results indicate some influence of ET on foals' growth since milk's composition on both groups (G-NM and G-ET) was similar ($P > 0.05$), as well as dietary and sanitary handling that foals and mares were submitted to.

It is a fact that one's phenotype is determined not only by its genotype, there is evidence that environmental modification during intra uterine period, as well as extra uterine environment may affect epigenetic features [18,24]. Researchers from England observed a variety of organs and tissues that are programmed and reprogrammed in uterus with several consequences on their physiology, and these disorders produced by intra uterine challenges can be transmitted to the next generation [4]. Therefore, these studies are relevant to analyze the reproductive biotechnology consequences in order to improve these methods efficiency and reduce possible deleterious effects.

Researches using Thoroughbred horses and Shetland ponies demonstrated considerable influence of mothers' size and weight on various biometric indexes of the respective foal [1,2]. These authors presented that foals' weight at birth is initially determined by chorioallantoic microscopic total surface since uterus' size is directly related to mare's size. It is further demonstrated that, when foals are produced by intra uterine delay pattern, they have a growth rate reduction mainly on the first 20 weeks of age and are reduced in later stages, proving that intra uterine environment can show its effects during postnatal development. Nonetheless, it should be noticed that the authors analyzed breeds with 200 kg difference in weight between the individuals on their studies, which did not occur in the present experiment, which may have contributed to clear variation detection on foals' development causing growth delay. In the present study, the contrary occurred, ET foals' exhibited greater development at the end of the period under review, showing greater corporal mass and cannon bone girth.

Studying thyroid's hormones (T3 and T4) concentrations and glucose homeostasis on foals submitted to intra uterine delay [14], observed T4 concentration reduction immediately at post partum and T3 level reduction at birth to weaning, on Pony foals that grown in heavy mares and also observed important glucose metabolism disorders. In this experiment, glucose metabolism wasn't evaluated but it is known that rapid and early development foals, as shown on ET group, can present glucose function disarray and therefore develop some degree of osteochondrosis [17], causing a chondrocyte maturation defect [15]. For this reason, technicians should stimulate growth within each breed parameter, since either growth delay or acceleration may lead to locomotor illness and so compromising horse future athlete performance.

Changes of physical and skeleton progress patterns on suckling foals remained after weaning. However it could be observed that the present study foals had their development under proposed parameters [8], which affirm that animals have to duplicate their birth weight at the first month of age and should be five times higher at weaning. It stands out that ET treatment foals reported slightly high values indicating this research and future studies relevance to assure if growth beyond observed commonly is beneficial or damaging.

By tracking different breed foals' progress, at tropical conditions in Brazil [19], reported that the animals duplicated their BM on the first 40 days of age, and also the total weight gain, from birth until 160 days of age, was 124 kg. In the present research the gain was 115 kg for NM foals and 138 kg for ET animals, therefore, the later presented higher pondered gain. Recently, this experiment researcher group [7], analyzed foals' development in the same handling conditions, where animals were free to exercise and had same nutritional plan, they found very similar values if compared to the current study indicating that these figures are appropriate to evaluate Quarter Horse foals raised at tropical conditions.

Mare's milk is the main nutritional source for the offspring [12], therefore foals' growth, especially on the first weeks of age, depends almost completely on the mother. It is also known the relevance of bioactive components as lysozymes, lactoferrins, immunoglobulin, long chain fatty acids (omega 3) and fat-soluble vitamins present in mares' milk [10]. For this reason, it can be named functional food, preventing against metabolic diseases and mammals' chronic disorders [11].

In this experiment it wasn't observed milk's composition variation between both groups of mares, but mare's colostrum showed different protein and lactose levels. Protein percentage was higher on the ET mares' colostrum and this group presented lower lactose percentage when compared to NM mares' group. It is important to emphasize that all mares were submitted to the same nutritional plan. It is necessary to identify variables, which may have influenced the colostrum composition between the two groups.

In 1996, Ullrey *et al*. [23] studied Arabian and Quarter Horse mares milk's composition and reported that protein and fat contents decrease their levels during lactation while lactose content increases. Similar results were found on Quarter Horses mares [5]. Studies using other horse breeds demonstrated similar change patterns on milk's composition during lactation [3,20,21]. All these concluding remarks were similar to the present experiment.

An important comment about artificial reproductive methods is the Large Offspring Syndrome (LOS) occurring in cloned ovine and bovine newborns [6,26,27] characterized by increased birth weight and health risks to the neonates. In humans, there is epidemiologic evidence that assisted reproduction techniques indicates the risk, although there is a rare occurrence of imprinting disorders as related in children in Netherlands, produced by several types of manipulation during fertilization [13]. It is also known that a wide range of pathology observed at human adult life is associated with the beginning events of life [14]. It was used different reproduction techniques from this study but still emphasizes the influence of artificial reproductive methods on the following generations.

The present experiment compared reproduction methods influence (NM and ET) on suckling foals' development and it was possible to observe the effects complexity, since weren't no effects of these methods on the birth weight. However, this effect appear at the second month of age showing a start on information emergence signalizing that theses consequences must be better studied so one can improve health conditions to the next generations. This study was conducted at a commercial farm where foals are negotiated since their first year, which made impossible to get information about their adult life.

CONCLUSION

In summary, there is little influence of the reproductive method over the lactating foal's development when we compered two groups of foals obtained from natural mate and embryo transfer. However, foals from embryo transfer had large body mass at weaning when compared with natural mate group. Finally, the mares' milk composition was similar between both groups of mares used in this study.

MANUFACTURERS

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