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REPRODUCTIVE FUNCTION IN PUREBRED ARABIAN STALLIONS AS RELATED TO THE LEVELS OF CHEMICAL ELEMENTS IN MANE HAIR SAMPLES

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Abstract

In modern animal husbandry, along with the improvement of reproductive technologies, it is becoming increasingly important to restore the natural fertility of livestock, in particular breeding producers. Reproductive function is one of the most sensitive that reacts to changes in biogeochemical and environmental parameters. In this regard, research aimed at studying effects of the level of chemical elements in the body on reproductive function in stallions is rather important. The parameters of sperm quality depending on the level of chemical elements in hair sampled from mane were studied. In this work we used biomaterial of purebred Arabian stallions (n = 50) of the same biogeochemical province. Reproductive qualities of stallions were evaluated in relation to the pool of chemical elements rated by concentration in the mane hair. All animals were grouped according to the levels of individual elements in hair (i.e. up to percentile 25, within the interval of percentiles 25-75 and above percentile 75). Also the quality of fresh and cryopreserved semen was estimated. Hair profiles was determined for 25 elements (Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, I, K, Li, Mg, Mn, Na, Ni, P, Pb, Se, Si, Sn, Hg, Sr, V, Zn) by inductively coupled plasma atomic emission and mass spectrometry (ICP-AES and ICP-MS). Ejaculate was assessed for volume, concentration, total spermatozoa number, number of spermatozoa with progressive motility and semen viability. The validity of the differences was verified by Mann-Whitney U test. It has been found out that the mane hair analysis can be used to detect reduced fertility in stallions. Increase in average values of Sr to 4.19±0.12, Se to 0,559±0.015, B to 21.55±1.14 μg/g in hair results in decrease in number and activity of sperm and filtrate volume. Activity and survival of stallion spermatozoa after thawing was in inverse correlation with Pb and Sn pool in the body, as determined by element level in the mane hair. With the increase in the concentration of these elements to 0,806±0206 and 0,051±0.008 mg/g, the sperm activity declines after thawing by 3.8-7.7 % and survival by 26.4-29.5 %, respectively. Animals with copper, silicon and vanadium in hair less than that of percentile 25, had the most active sperm. The survival rate of fresh sperm was associated with V concentration in hair being the highest in animals with low levels of this mineral. Thus, the assay of mane hair mineral profile can be used in monitoring for timely correction of stallions' fertility.

Keywords: purebred horses, Arabian breed, stallion, profiles of mineral elements in the horse hair, reproductive function, sperm quality

At the present time, both new reproductive technologies and methods to restore the natural fertility of livestock, including horses, are relevant. In addition to the endogenous factors (parental diseases, infections, hereditary diseases), which can cause reproductive health disorders, the conditions of biogeochemical province and environmental parameters [1] hold a significant position, as well as mineral provision of animals. Chemical elements are important ecophysiological agents which determine the morphofunctional characteristics of the reproductive system [2-4].

Elements which are directly affecting the qualitative characteristics of semen can be divided into several groups: essential macroelements (Na, K, Ca, Mg, etc.), trace elements needed for normal sperm functioning (Zn, Cu, Mn, Co, Se) [5], and toxic elements (Pb, Cd, Hg, etc.) which are extremely harmful and dangerous even in small amounts [6-8].

Horse semen evaluation before the breeding period does not allow us quickly and efficiently to level individual deviations in the contents of chemical elements through the optimization of animal feed. Because of the relative labor intensity and injurious risk of sperm taking, an early assessment of its elemental status using some other biomaterial is preferable. Examination of the elemental hair composition is quite widespread in medicine [9]. This noninvasive method makes possible to assess animal health status, to exclude pathologies caused by heavy metals intoxication, and to detect disorders in animal nutrition [10].

This paper is the first report on the connection between some chemical element contents in the stallion's mane hair and sperm quality and cryoresistance. We found an inverse relationship between the strontium, selenium, boron accumulation and ejaculate volume, the quantity and motility of spermatozoa, and also between lead and tin content and the motility and viability of spermatozoa after thawing. Animals with a low copper, silicon and vanadium hair content had the highest sperm motility.

Our aim was to study reproductive function of purebred stallions depending on their mineral status, as estimated by mane hair elemental analysis.

Techniques. The research was carried out on 4-5 year old purebred Arabian stallions with body weight of 410.5±25.3 kg (Terskys Stud farm No.169, Stavropol'skiy Krai, 2016-2017). Animal keeping and experiments were in accordance with the instructions and recommendations of the USSR Ministry of Health Order No. 701 of July 27, 1978 "On the introduction of amendments to the Order of the Ministry of Health USSR of 12.08.77 No. 755" and "The Guide for Care and Use of Laboratory Animals" (National Academy Press, Washington, D.C. 1996). All efforts had been made to minimize animal suffering and to reduce the number of samples in the study. The selected experimental group contained clinically healthy stallions (n = 50). Mane hair, fresh and cryopreserved sperm served as a biomaterial. The samples of cryopreserved sperm were obtained from the Biosphere Collection "Cryobank of genetic resources" (All-Russian Research Institute for Horse Breeding).

To estimate reproductive qualities depending on the pool of chemical elements, the content of which was determined in mane hair, the stallions were grouped by the concentration of elements in the hair: to the 25th percentile, within the 25th to 75th percentiles, higher than the 75th percentile. Percentile intervals were based on previous reports on human biosubstrates, where the physiological norm in the population was identified as the 25th to 75th percentiles [11. Daily food ration of the test animals during 2 months prior to the biomaterial collection contained 75.3 g of Ca, 52.8 g of P, 14.3 g of Mg, 1211.8 mg of Fe, 128.1 g of Cu, 7.2 mg of Co, 604 mg of Mn, and 7.4 mg of I.

The elemental composition was analyzed by inductively coupled plasma atomic emission and mass spectrometry (ICP-AES and ICP-MS). A microwave digestion system, model SW4 Speedwave (Berghof, Germany) was used for the digestion of samples. In the obtained ash, 25 elements (Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, I, K, Li, Mg, Mn, Na, Ni, P, Pb, Se, Si, Sn, Hg, Sr, V, Zn) were determined using an Elan 9000 mass spectrometer (Perkin Elmer, USA) and an Optima 2000V atomic emission spectrometer (Perkin Elmer, USA).

Semen was collected using an artificial vagina in response to a mare in heat (All-Russian Research Institute of Horse Breeding, ARRIHB) (not less than 5 ejaculates from each animal) every 48 h. The sperm indicators of the first two ejaculates after the period of sexual dormancy were not used in the processing. Each ejaculate was initially evaluated by the following indicators: volume, concentration, total number of spermatozoa (TNS), total number of progressively motile sperm cells (TNS PM), and sperm survival at 2-4 °C. The samples were diluted with a lactose-chelate-citrate-yolk (LCCY) medium in a volume ratio of 1:3. The sperm were frozen in liquid nitrogen vapors using the ARRIHB technology. Frozen sperm were stored in liquid nitrogen at 196 °C. The thawing was carried out in water bath at 40 °C. After thawing cryopreserved sperm, the viability of spermatozoa at 2-4 °C and progressive motility were determined.

The Shapiro-Wilk test was used to verify the hypothesis of normal distribution of other quantitative traits. Hence distribution of the investigated numeric indicators differed from the normal one, the validity of differences was checked by the Mann-Whitney U test. In all procedures for statistical analysis, significance level (P) was calculated, the critical significance level taken was less than or equal to 0.05. The tables show the average values of the indicators (*M*) and their standard errors (±SEM). For data processing, the Statistica 10.0 software was used (StatSoft, Inc., USA).

Resultes. A comparative evaluation of the stallion mane hair composition identified significant differences in an amount of certain chemical elements in animals of different groups (Table 1).

1. Chemical element contents (mg/g) **in the mane hair of purebred Arabian stallions depending on the percentile range** (*M*±SEM, Terskii Stud farm No. 169, Stavropol'skiy Krai, 2016-2017)

Element	Percentile interval		
	< 25	25-75	> 75
	Macroelements		
Ca	500.100±21.2400	839.000±44.9100***	1223.000±61.0800***
K	275.100±16.0000	527.400±28.6000***	1248.000±167.8000***
Mg	160.300±21.1200	372.300±22.2800***	632.500±27.3900***
Na	94.560±4.2200	149.500±4.8800***	286.100±38.5500***
P	249.200±55.3540	496.200±19.6000***	765.300±29.7230***
	Essential microelements		
Co	0.010±0.0007	0.020±0.0010***	0.079±0.0130***
Cr	0.043±0.0040	0.130±0.0140***	0.514±0.1510**
Cu	4.760±0.1100	5.670±0.1250***	7.800±0.5800***
Fe	25.630±1.8100	48.760±3.2900***	168.200±24.7500***
I	0.018±0.0040	0.062±0.0060***	0.232±0.0570**
Mn	0.555±0.0370	1.030±0.0630***	3.830±0.5350***
Se	0.278±0.0130	0.395±0.0130***	0.559±0.0150***
Zn	126.300±3.0800	146.900±2.4900***	181.900±4.3200***
	Conditionally essential microelements		
B	1.540±0.1369	7.050±1.1200**	21.550±1.1400***
Li	0.049±0.0060	0.118±0.0065***	0.249±0.0270***
Ni	0,082±0,0030	0,123±0,0090***	0,441±0,0630***
Si	2,900±0,3110	5,700±0,2850***	8,780±0,6660***
Sr	1,0600±0,1002	2,500±0,1780***	4,190±0,1200***
V	0,028±0,0030	0,072±0,0070***	0,239±0,0270***
	Toxic elements		
Al	7.980±0.7580	24,150±2,2700***	79,800±13,4000**

Continued Table 1

As	0.016±0.0007	0,024±0,0008***	0,058±0,0100***
Cd	0.002±0.0002	0,004±0,0003**	0,010±0,0010***
Pb	0.027±0.0010	0,061±0,0070	0,806±0,2060*
Sn	0.003±0.0001	0,009±0,0010**	0,051±0,0080***

*, **, *** P < 0.05; P < 0.01; P < 0.001, respectively, compared to the group of a percentile interval < 25.

Filtering ejaculates of animals having different hair chemical composition revealed that the ejaculate volume dependes on the amount of zinc, boron, phosphorus, lead, selenium and strontium (Table 2).

2. Ejaculate volume after filtration (ml) **in the groups of purebred Arabian stallions depending on the percentile range of chemical element concentration in mane hair** (*M*±SEM, Terskii Stud farm No. 169, Stavropol'skii Krai, 2016-2017)

Element	Percentile interval		
	< 25	25-75	> 75
As	30.51±6.730	36.84±21.260	43.25±23.240
B	51.40±17.690	35.62±20.250	25.90±9.760**
Cu	35.99±11.830	38.30±7.990	35.57±12.700
Fe	38.72±9.910	34.13±8.060	41.77±13.380
Li	45.56±12.820	37.34±13.810	28.06±11.830
Na	46.00±8.610	30.61±3.010*	42.02±9.950
P	61.97±6.950	25.84±1.970***	36.29±5.880**
Pb	25.77±3.790	32.06±4.070	55.57±6.040*
Se	53.69±6.520	35.33±5.100*	24.24±1.930***
Si	28.19±5.000	40.68±10.600	38.27±13.600
Sn	40.67±12.770	35.24±6.650	37.17±18.070
Sr	58.71±6.120	33.77±4.350**	22.54±1.290***
V	38.73±9.910	32.75±8.370	44.71±11.300
Zn	28.74±3.560	32.76±4.230	54.69±8.880*

*, **, *** P < 0.05; P < 0.01; P < 0.001, respectively, compared to the group of a percentile interval < 25.

The mane hair mineral content reflects exposure of elements from the diet [12] and is linked, as a whole, to the value of their exchange pool in the body [13]. Close linkage between the elemental state of stallion and their reproductive system functioning is identified [14, 15], which in turn, is determines by the effect of elements on mammals' spermatogenesis and sperm quality [16-18].

We found a 66.9-90.3 % increase in the total filtrate volume as the zinc content in mane hair grew, which complied with the earlier findings [19] and can be explained by important role of zinc in the normal functioning of the prostate and the reproductive system as a whole [20, 21]. The physiological importance of zinc, which contains in the prostate secretion, is to implement mechanism of the sperm head and tail separation, as well as the chromatin decondensation. Zinc in seminal plasma stabilizes the cell membrane and nuclear chromatin of spermatozoa [22, 23]. This element also plays an important regulatory role in condensation and acrosome reaction [24)]. However, it is difficult to explain the significant (almost twice) decreasing in ejaculate volume related to elevation of boron level. Negative effects of growing dietary boron on the volume and sperm composition were not detected earlier [25].

An ejaculate volume decreased as the amount of hair strontium, a conditionally essential element, increased. An increase in Sr content from the minimum to the maximum within the 25-75 and > 75 percentile intervals resulted in a decrease in the ejaculate volume by 73.9 % (P < 0.01) and 49.2 % (P < 0.001), respectively. A similar trend was noted for the effect of strontium on the TNS index, which, on the whole, indicates a negative effect of this element on the stallion reproductive ability. However, it is difficult to interpret the obtained data without information about the physiological norm of strontium as a component of the elemental status. Perhaps the population we have formed is characterized by an increased pool of this element. Meanwhile, strontium is capable to demonstrate unique properties for the activation of oocytes, followed by stimulation of their development to the 8-cell or blastocyst stages [26-28]. This can be used to increase the fertilizing capacity of spermatozoa and, as a result, effective reducing amount of single dose.

On the contrary, the filtrate volume increased by 115.6-73.3 % ($P < 0.05$) as hair lead level rising. In whole, a high exchange lead pool is linked to the infertility caused by the induction of a spontaneous premature acrosome reaction, and an increase of this element concentration in the seminal plasma can adversely affect the fertility potential of sperm in vitro [29].

It must be noted that the physiological standards is applicable only to essential and macroelements, but not to toxic elements. The physiological standard lies within the 25th-75th percentiles, and the values above and below this percentile interval for vital elements can be considered as abnormal. Despite the fact that the minimum ejaculate volume (25.84 ml) was recorded in the group with the phosphorus content corresponding to the physiological norm (25th-75th percentiles), the sperm concentration in fresh semen here was maximum (268.2 million/ml) .

3. Quality indicators of diluted ejaculates in the groups of purebred Arabian stallions depending on the percentile range of chemical element concentration in mane hair (*M*±SEM, Terskii Stud farm No. 169, Stavropol'skii Krai, 2016-2017)

Element	Percentile interval					
	< 25		25-75		> 75	
	A	B	A	B	A	B
As	50,57±8,630	128,2±38,14	47,97±7,930	121,8±28,67	47,05±6,290	132,7±16,49
B	47,34±6,250	129,4±14,63	48,71±9,450	127,9±32,52	48,57±4,030	120,1±30,24
Cu	53,38±2,410	131,9±19,47	46,80±2,160	128,8±29,51	46,63±1,740*	115,7±32,62
Fe	52,14±6,120	145,4±6,56	47,78±8,690	115,1±18,33	45,70±5,130	131,5±4,78
Li	50,71±10,530	130,2±25,28	47,18±6,420	120,9±33,24	48,48±6,980	134,3±16,61
Na	49,57±9,620	126,8±15,79	48,20±7,690	124,4±13,85	47,42±5,680	130,0±17,20
P	46,57±5,560	125,1±13,60	49,38±9,140	127,9±22,52	47,91±5,740	124,2±21,50
Pb	50,33±7,850	135,3±14,04	49,95±1,460	126,1±11,35	43,14±2,400	123,4±16,56
Se	45,28±7,400	128,5±20,45	49,69±8,580	125,9±30,29	48,52±4,850	125,1±33,04
Si	54,95±2,950	124,9±35,05	47,89±1,570*	131,5±28,99	42,71±2,060**	116,9±17,23
Sn	48,71±8,130	131,9±13,90	49,00±5,810	130,5±16,70	46,87±10,230	114,3±23,03
Sr	48,00±5,650	133,7±7,56	48,22±8,300	120,5±13,44	48,95±8,500	131,7±13,65
V	52,14±2,310	145,4±6,56	48,38±2,070	118,5±8,45	44,49±2,490*	124,3±6,24*
Zn	51,95±7,340	133,8±15,39	48,16±6,570	125,9±16,22	45,14±5,550	120,0±16,00

Note. A — motility, %; B — viability, h.
*, ** $P < 0.05$; $P < 0.01$, respectively, compared to the group of a percentile interval < 25.

Animals with a low content of copper, silicon and vanadium in the hair had the most active spermatozoa (Table 3). Exceeding, when compared to the stallions with large (> 75th percentile) and mean (25th-75th percentiles) quantities of these elements, was 6.8 ($P < 0.05$) and 6.6 % for copper, by 12.3 ($P < 0.01$) and 7.1 % ($P < 0.05$) for silicon, and by 7.3 ($P < 0.05$) and 3.7 % for vanadium, respectively. Several studies have found an inverse relationship between high copper content and sperm quality [30, 31]. The copper pool is also associated closely with the activity of copper-containing enzymes [32].

The viability of diluted semen, when stored at 2-4 °C, depended on the vanadium concertation and was the highest in animals with a low (<25th percentile) level of this element, that is, by 17.0 % ($P < 0.05$) and 22.7 % higher compared to stallions with high and medium hair vanadium level, respectively.

Superficially, unordinary changes in the reproductive ability of stallions depending on the value of the selenium pool were identified. In particular, as the amount of selenium in the hair increased from 0.278±0.013 to 0.395±0.013 and 0.559 0.015 μg/g, the concentration of spermatozoa in the ejaculate decreased by 39.2 % ($P < 0.05$) and 18.1 % ($P <0.05$). There was a reduction in the sperm motility. It is appreciable that selenium deficiency has a very negative effect on animal fertility [33]. According to it, while increasing dietary Se, an improvement in stallion sperm quality should be expected. However, the information about the limits of the element content in the mane hair is absent. Perhaps, examined boundaries exceeded the physiological rate. At the same time, there are data which shows the ambiguous effect of selenium additives on reproductive qualities [34, 35]. In some studies, selenium supplementation did not change semen amount and quality [36]. We observed a reduction in the amount of active sperm in the ejaculate with the increasing of hair selenium content. A similar result was obtained for boron, lithium, and strontium (Table 4).

4. Amount of active spermatozoa in the ejaculate (billions) **of the purebred Arabian stallions depending on the percentile range of chemical element concentration in mane hair** (*M*±SEM, Terskii Stud farm No. 169, Stavropol'skii Krai, 2016-2017)

Element	Percentile interval					
	< 25		25-75		> 75	
	TNS	TNS PM	TNS	TNS PM	TNS	TNS PM
As	6.83±2.540	3.60±1.570	7.88±3.550	3.82±1.960	9.14±2.350*	4.26±1.030
B	9.75±2.750	4.66±1.720	8.08±3.210	3.98±1.660	5.97±1.890*	2.93±1.070*
Cu	8.13±2.940	4.36±2.170	8.13±1.810	3.84±1.490	7.48±2.040	3.50±1.410
Fe	8.72±1.970	4.51±0.890	7.02±3.040	3.41±1.620	9.28±2.640	4.30±1.040
Li	9.58±0.240	4.72±0.630	7.41±3.770	3.59±0.520	7.58±1.760	3.69±0.400*
Na	9.81±2.260	4.76±1.080	7.19±2.210	3.58±1.870	7.81±2.990	3.67±1.350
P	9.53±0.920	4.36±0.900	6.78±0.710*	3.44±1.620	8.98±3.570	4.36±2.090
Pb	5.98±2.110	3.33±2.340	7.31±1.620	3.68±1.460	10.63±1.910	4.68±1.750
Se	10.5±0.970	4.78±0.630	7.54±0.830*	3.83±0.450	6.38±0.500**	3.11±0.310*
Si	6.81±1.360	3.78±1.040	8.82±2.410	4.27±1.860	7.33±2.290	3.17±1.470
Sn	7.21±2.650	3.60±1.840	8.25±1.930	4.09±1.740	8.16±2.030	3.77±1.370
Sr	10.79±0.870	5.24±0.590	7.75±0.690*	3.71±0.350*	5.64±0.900**	2.91±0.580*
V	8.72±1.970	4.51±0.900	6.74±1.930	3.34±1.640	9.88±2.300	4.43±1.930
Zn	7.45±1.780	3.83±1.030	7.74±1.520	3.83±1.990	9.01±2.120	4.07±1.410

N o t e. TNS — total number of spermatozoa, TNS PM — total number of progressively motile sperm cells.
*, ** P < 0.05; P < 0.01, respectively, compared to the group of a percentile interval < 25.

Thorough analysis of cryopreserved sperm quality associated with concertation of chemical elements in mane revealed the dependence of viability and sperm motility only on the lead and tin content. In particular, with a maximum increase in the amount of lead in the hair, there was a decrease in the semen motility after thawing from 27.66 to 20.00 % (P < 0.05) (Table 5). A similar decrease in the semen viability after thawing from 78.4 to 48.9 h (P < 0.05) with a high tin content was noted when compared to the > 75 and < 25 percentile intervals.

5. Quality of thawed semen of the purebred Arabian stallions depending on the percentile range on the content of chemical elements in the mane hair (*M*±SEM, Terskii Stud farm No. 169, Stavropol'skii Krai, 2016-2017)

Element	Percentile interval					
	< 25		25-75		> 75	
	A	B	A	B	A	B
As	23.57±5.160	67,1±13,00	23,07±5,630	64,6±14,55	23,37±2,720	77,8±17,05
B	22.85±3.020	69,6±14,20	24,09±5,730	71,1±55,99	21,95±7,720	62,9±17,61
Cu	26.91±4.550	78,6±17,69	22,74±5,720	67,8±32,84	20,78±5,400	61,1±14,00
Fe	26.00±5.250	76,5±14,66	22,06±5,290	63,3±16,63	23,17±2,570	72,8±16,35
Li	26.42±5.390	70,3±17,21	22,27±6,750	68,4±15,6	22,28±3,450	68,1±11,66
Na	26.50±6.530	73,3±17,62	21,97±8,580	68,7±11,47	22,85±3,800	64,5±11,95
P	23.92±2.970	67,7±15,67	24,37±3,710	70,7±15,75	20,28±4,150	65,8±10,35
Pb	27.66±3.180	67,5±25,23	23,79±2,040	72,8±33,28	20,00±1,780*	58,3±21,27
Se	21.00±5.710	68,1±15,14	25,16±4,570	71,7±11,41	21,51±5,440	63,1±14,30
Si	26.41±5.980	70,5±12,63	23,20±7,290	74,0±12,04	20,31±5,240	55,9±19,09
Sn	26.14±4.220	78,4±7,18	23,42±6,810	75,3±8,56	20,52±11,560	48,9±9,55*
Sr	23.57±2.570	75,4±14,24	22,51±5,700	64,4±16,05	24,62±4,040	71,5±15,65
V	26.00±5.250	76,1±14,66	23,07±6,760	66,2±14,98	21,00±5,410	66,6±14,79
Zn	26.8±5.630	74,5±18,15	22,76±6,320	68,8±20,68	20,85±6,260	63,0±23,24

N o t e. A — motility, %; B — viability, h.
* P < 0.05 compared to the group of a percentile interval < 25.

To date, extensive experimental data of toxic element effects on sperm quality and fertility has been accumulated. The action of toxic elements is multiple and manifests itself in reducing sperm concentration [37] and suppressing sperm motility [38]. Significant negative correlations have been established between lead concentration in sperm, motility (r = –0.65, P < 0.001) and sperm viability (r = –0.62, P < 0.001) [39].

Thus, the mane hair elemental analysis can be used for the reduced stallion fertility identification. Increasing content of strontium up to 4.1±0.12, selenium up to 0.559±0.015, boron up to 21.55±1.14 μg/g in the hair is connected with a decrease in ejaculate volume, quantity and spermatozoa motility. The motility and viability of stallion spermatozoa after freezing are inversely related to the lead and tin levels in the body. With an increasing the tin content in the hair up to 0.806±0.206 μg/g, the progressive spermatozoa motility after thawing decreased from 27.66±3.18 to 20.00±1.78 %, or by 7.7 % on average). With an increasing the lead concetration in the hair up to 0.051±0.008 μg/g, the viability of spermatozoa after thawing reduced by 29.5 h, or by 37.6 %. The highest spermatozoa motility was characteristic of animals with low (less than 25th percentile) copper, silicon and vanadium levels in hair. The viability of fresh semen correlated with the vanadium content in the hair and was the largest in animals with a low content of this element. Further work involves the determination of reference and percentile ranges for the content of chemical elements in mane hair in connection with the pool of these elements in the body.

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