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Original study

Environmental effects and repeatability estimates for sperm production and semen quality of Holstein bulls

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Abstract

The purpose of this study was to estimate environmental effects on semen production of artificial insemination (AI) Holstein bulls managed under Moroccan conditions. A total of 4 046 ejaculates collected from 34 Holstein bulls in the years 2009-2013 were analysed. Studied variables were volume, concentration, total number of spermatozoa, mass motility, individual motility and post-thawing motility. Data were analysed by REML method using the mixed model including the random effect of bull and the fixed effects of age at collection, season of collection, year of collection, interval between two collections and ejaculate order. The effect of age of bulls was significant for all studied variables, except for mass motility. Spring and winter were the best seasons for sperm production and quality. Bulls' ejaculates collected once a day at one day interval produced monthly 30 %, 86 %, 156 %, 183 % and 185 % more motile spermatozoa than those collected once a day at 2, 3, 4, 5 and 6 days interval, respectively. Moreover, bulls' sperm collected twice a day at 1, 2, 3, 4, 5 and 6 days interval produced monthly (sum of motile spermatozoa obtained at 1st and 2nd ejaculates) 77 %, 70 %, 65 %, 68 %, 84 % and 91 % more than those collected once a day at the same interval, respectively. Repeatability estimates for semen traits were medium to high. They varied from 0.157 for mass motility to 0.411 for ejaculate volume. It was concluded that environmental factors clearly contribute to semen production in Holstein bulls and short intervals between collections and two collections per day are maximising sperm production.

Keywords: ejaculate, semen, spermatozoa, artificial insemination, volume, motility, repeatability

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Introduction

Moroccan dairy herds are experiencing a rapid expansion of artificial insemination (AI). Although less than 10 % of the national cow herds are artificially inseminated, the number of AI has increased during the last two decades by about 31 % per year. In Morocco, AI concerns mainly the improved dairy cows, with Holstein AI representing more than 68 % (Boujenane 2002). Also, there are two AI centres in the country that are managed by the government. Their production does not cover the increased AI demand; therefore thousands of frozen doses are imported annually from European and North-American countries. Recently, one AI centre was transferred to a breeders' association, which hopes to increase the local production in order to satisfy the breeders' AI demand. Moreover, most of the bulls used in the centre have been imported from Europe, i.e. developed in temperate areas. Thus, there is a need for extensive knowledge of factors affecting sperm production and semen quality obtained under Moroccan management conditions in order to increase the productivity and profitability of the AI centre. Environmental factors that affect semen production are mainly age of the bull at semen collection, frequency of collection and season of collection, the latter e.g. including food quality, ambient temperature, humidity or day length (Everett & Bean 1982, Taylor *et al.* 1985, Mathevon *et al.* 1998, Karoui *et al.* 2011). Indeed, with the knowledge of these factors, the centre might adapt management of AI bulls to improve semen production.

The purpose of this study was to estimate effects of age, season, year and frequency of collection on semen characteristics and repeatability for semen traits of AI Holstein bulls managed and housed under Moroccan conditions.

Material and methods

Animals and management

Semen data from a Moroccan AI centre, CRIA Fouarat, collected in the years 2009-2013 were evaluated. Average daily ambient temperature during the collection period was 16.3 °C with a minimum of –3.7 °C and a maximum of 42.8 °C. Average humidity was 77 %. A total of 4 046 ejaculates were analysed. They were obtained from 34 Holstein bulls of which 30 were imported from France and four were born in Morocco. Age of bulls at collection averaged 39.5 month, varying from 13 to 93 months. The bulls were managed uniformly. They were individually housed and maintained on a diet of mixed alfalfa and oat hay, approximately 500 g of concentrate and vitamins and minerals mix. All the bulls are periodically vaccinated against foot and mouth disease.

Semen collection

In general, semen was routinely collected in the centre three times a week (Monday, Wednesday and Friday) at early morning. Semen collection was preceded by a controlled sexual preparation. Thus, after arrival on the collection floor, bulls were tied in the waiting stall from which they observed the collection of the other bulls. When their libido was stimulated, they were allowed a short walk and two false mounts with a 2-min interval. At the third mount using a teaser animal (a bull known for its docility and robustness), the ejaculate was obtained with an artificial vagina (37 °C). One or two ejaculates were collected from each bull on each collection day. Ejaculates obtained from the 1st and 2nd collection represented 60.8 % and 39.2 %, respectively. The interval between two consecutive collections varied from 1 to 36 days, with an average of 3.33 days.

Semen exam

Immediately after collection, the tube of semen was placed in a water bath at 32 to 34 °C. Each ejaculate was evaluated for volume, sperm concentration and motility traits. Some ejaculates were discarded by the technician on either established standards or judgment (colour, presence of blood or dirt…). Volume was measured in the graduated collection tube (ml). Sperm concentration (spermatozoa number per ml) was estimated by using a photometer (Reference 1203, IMV Technologie, France). Mass motility score was subjectively assessed for undiluted unstained semen using a scale from 0 (no activity) to 5 (rapid swirling motion). Individual ejaculates were then diluted with egg yolk citrate-glycerol extender to give a concentration of 24 million total spermatozoa per 0.25 ml straw. Individual motility or percent progressive motility, expressed by the percent of mobile spermatozoa per ejaculate, was estimated in 5 % steps on a subjective scale of 0 % to 100 % by examining unstained diluted semen under microscope (Nikon Eclipse 50i, IMV Technologie, France) using a 100× magnification. After gradually cooling to 4 °C, the semen was packaged in 0.25 ml straws (24 million motile spermatozoa before freezing per straw) and frozen at −196 °C. Post-thawing motility was measured 24 hours after storage in liquid nitrogen. For post-thaw semen evaluation, two straws from each bull at each collection were thawed in a water bath of 37 °C for 30 seconds and evaluated individually for the percentage of progressively motile spermatozoa. Ejaculates with less than 35 % of post-thawing progressively motile spermatozoa were eliminated. Valid straws were stored in large containers containing liquid nitrogen.

Statistical analyses

The traits studied were ejaculate volume, sperm concentration, mass motility score, individual motility, post-thawing progressive individual motility and total number of spermatozoa per ejaculate, which was calculated as the product of ejaculate volume and sperm concentration. After editing, the file analysed included 4 046 ejaculates corresponding to 34 bulls. The number of ejaculates of each bull averaged 119, varying from 7 to 349 ejaculates. Except mass motility score that was analysed using the GLIMMIX (SAS Institute Inc., Cary, NC, USA) procedure (SAS 2002), since it is multinomially distributed, the other semen traits (ejaculate volume, sperm concentration, total number of spermatozoa per ejaculate, individual motility and post-thawing individual motility), which are normally distributed, were analysed using the MIXED procedure (SAS 2002). The mixed model used to analyse all variables included the random effect of bull (34 bulls) and the fixed effects of age at collection (7 levels: age≤24 mo., 24<age≤30 mo., 30<age≤36 mo., 36<age≤42 mo., 42<age≤48 mo., 48<age≤54 mo., age>54 mo.), season of collection (4 levels: winter: January – March, spring: April – June, summer: July – September, autumn: October – December), year of collection (5 levels: 2009, 2010,…, 2013), interval between two successive collections (6 levels: 1, 2, …, 6 or greater) and ejaculate order

(2 levels: 1st and 2nd). Interactions between effects were assumed negligible and hence were not tested. When the effect was determined to be significant (P<0.05), differences among least-squares means were examined by the Tukey method for multiple comparisons.

Repeatability estimates for semen traits were calculated by using the following formula:

$$\frac{\sigma^2 bull}{\sigma^2 bull + \sigma^2 error} \quad (1)$$

The variance components $\sigma^2 bull$ and $\sigma^2 error$ were estimated from the MIXED procedure (SAS 2002).

Results and discussion

Descriptive statistics

Arithmetic means, standard deviations and coefficients of variation of sperm characteristics of Holstein bulls' semen collected in Moroccan conditions are reported in Table 1. They are in general similar to those found in other studies (Everett & Bean 1982, Taylor *et al.* 1985, Mathevon *et al.* 1998, Karoui *et al.* 2011). Moreover, ejaculate volume had the highest and individual motility had the lowest coefficient of variation.

Table 1
Number of observations, arithmetic means, standard deviations and coefficients of variation of sperm production and semen quality of Holstein bulls

Semen trait	Number	Arithmetic mean	Standard deviation	Coefficient of variation, %
Ejaculate volume, ml	4046	5.05	2.25	44.5
Sperm concentration, $\times 10^9$/ml	4046	0.95	0.21	22.1
Number of spermatozoa per ejaculate, $\times 10^9$	4046	4.90	2.63	53.7
Mass motility score (1-5 scale)	4046	3.58	0.50	14.0
Individual motility, %	4046	77.0	5.58	7.25
Post-thawing motility, %	4046	59.8	5.82	9.73

Systematic environmental effects

Age at collection

Age of bulls affects all semen traits (P<0.01 to P<0.001) except mass motility score (P>0.05) (Table 2). Ejaculate volume and total number of spermatozoa increased from the youngest to the oldest ages by 54.7 % and 33.5 %, respectively, whereas the largest difference for sperm concentration (10×109xml-1) was recorded between 24<age≤30 and 48<age≤54 classes. Also, individual motility and post-thawing motility differed significantly with age of bulls, but they showed a different pattern with a tendency to a slow decline as the age increased for ages greater than 54 months. These results are in agreement with those of most studies, which reported an increase in semen production and quality with age of bull (Everett & Bean 1982, Taylor *et al.* 1985, Mathevon *et al.* 1998, Brito *et al.* 2002, Fuerst-Waltl *et al.* 2006). Moreover, Mathevon *et al.* (1998) found that volume and number of spermatozoa per ejaculate tended to increase with age of the bull, regardless of season or interval between collections. It seems that the main factor, determining the total number of spermatozoa produced, is the increased ejaculate volume owing to testicular development, since the size

Table 2
Number of observations, least-squares means ± standard errors of semen production and semen quality of Holstein bulls[1]

Factor	Number	Ejaculate volume, ml	Sperm, concentratio $10^9 \times ml^{-1}$	Number of spermatozoa per ejaculate, 10^9	Mass motility score	Individual motility, %	Post-thawing motility, %
Age of bull at collection, months	***	***	***	ns	*	***	
Age≤24	682	3.86 ± 0.22^{a}	0.95 ± 0.02^{ab}	3.91 ± 0.25^{ac}	3.49±0.05	76.0 ± 0.65^{ab}	59.5 ± 0.69^{a}
24<age≤30	695	4.56 ± 0.21^{b}	0.97 ± 0.01^{a}	4.64 ± 0.23^{bc}	3.51±0.04	76.1 ± 0.59^{b}	58.9 ± 0.64^{a}
30<age≤36	668	4.81 ± 0.21^{bc}	0.95 ± 0.01^{ab}	4.80 ± 0.23^{bc}	3.43±0.04	75.4 ± 0.58^{ac}	59.0 ± 0.62^{a}
36<age≤42	435	5.05 ± 0.21^{cd}	0.93 ± 0.01^{b}	4.93 ± 0.24^{b}	3.47±0.05	75.6 ± 0.62^{ab}	59.6 ± 0.66^{a}
42<age≤48	500	5.00 ± 0.22^{c}	0.93 ± 0.02^{ab}	4.75 ± 0.24^{b}	3.44±0.05	74.8 ± 0.62^{cd}	57.6 ± 0.66^{b}
48<age≤54	482	5.44 ± 0.23^{d}	0.87 ± 0.02^{c}	4.54 ± 0.26^{c}	3.46±0.05	75.5 ± 0.67^{abc}	58.3 ± 0.71^{ab}
Age>54	584	5.97 ± 0.26^{e}	0.92 ± 0.02^{ab}	5.22 ± 0.30^{b}	3.43±0.06	74.3 ± 0.81^{d}	57.9 ± 0.84^{ab}

[1]Least-squares means within a column that do not have a common superscript (a-e) are significantly different ($P<0.05$).ns: not significant, $P>0.05$, *$P<0.05$, ***$P<0.001$

Table 3
Number of observations, least-squares means ± standard errors of semen production and semen quality of Holstein bulls1

Factor	Number	Ejaculate volume, ml	Sperm, concentratio $10^9 \times ml^{-1}$	Number of spermatozoa per ejaculate, 10^9	Mass motility score	Individual motility, %	Post-thawing motility, %
Season of collection		***	***	***	***	***	***
Winter	1 102	5.04 ± 0.21^{a}	0.99 ± 0.01^{a}	5.11 ± 0.23^{a}	3.47 ± 0.04^{ab}	75.4 ± 0.58^{a}	59.5 ± 0.63^{a}
Spring	898	5.09 ± 0.21^{a}	0.93 ± 0.01^{b}	4.76 ± 0.23^{b}	3.51 ± 0.04^{b}	76.4 ± 0.58^{b}	60.8 ± 0.62^{b}
Summer	653	4.78 ± 0.21^{b}	0.90 ± 0.01^{c}	4.36 ± 0.23^{c}	3.43 ± 0.04^{a}	74.6 ± 0.58^{c}	56.2 ± 0.62^{c}
Autumn	1 393	4.90 ± 0.20^{ab}	0.91 ± 0.01^{d}	4.51 ± 0.22^{c}	3.43 ± 0.04^{a}	75.1 ± 0.55^{ac}	58.2 ± 0.60^{d}
Year of collection		***	***	***	*	*	***
2009	957	4.18 ± 0.23^{a}	0.78 ± 0.02^{a}	3.09 ± 0.26^{a}	3.49 ± 0.05^{ab}	75.6 ± 0.68^{ab}	57.1 ± 0.72^{a}
2010	752	4.55 ± 0.22^{b}	0.75 ± 0.02^{b}	3.19 ± 0.25^{a}	3.44 ± 0.05^{ab}	75.4 ± 0.65^{ab}	58.6 ± 0.69^{bd}
2011	342	4.92 ± 0.22^{c}	1.21 ± 0.02^{c}	6.07 ± 0.25^{bc}	3.51 ± 0.05^{a}	75.2 ± 0.66^{ab}	61.6 ± 0.69^{c}
2012	1 637	5.40 ± 0.20^{d}	0.96 ± 0.01^{d}	5.34 ± 0.23^{b}	3.42 ± 0.04^{b}	74.8 ± 0.57^{b}	59.0 ± 0.62^{d}
2013	358	5.70 ± 0.23^{d}	0.96 ± 0.02^{d}	5.73 ± 0.26^{c}	3.45 ± 0.05^{ab}	75.9 ± 0.70^{a}	57.2 ± 0.73^{ab}

[1]Least-squares means within a column that do not have a common superscript (a-d) are significantly different ($P<0.05$). *$P<0.05$, ***$P<0.001$

of testes increases for at least five years after puberty (Amann & Almquist 1976). However, our results are not in agreement with those of Brito *et al.* (2002), who reported a non-significant effect of age on sperm concentration and motility.

Season of collection

The season at which semen was collected affects all semen traits of bulls ($P<0.001$). Semen characteristics were in general higher during spring and winter than during summer and autumn. Ejaculate volume, sperm concentration, total number of spermatozoa, mass motility score, individual motility and post-thawing motility of bulls' sperm collected in winter were 5.44 %, 10.0 %, 17.2 %, 1.17 %, 1.07 % and 5.87 %, respectively higher than those collected in summer. Many studies have reported on seasonal effects on semen production (Everett & Bean 1982, Mathevon *et al.* 1998), whereas other investigations failed to detect an effect of season (Brito *et al.* 2002). The present findings are consistent with those of Mathevon *et al.* (1998) who found higher values of semen characteristics during winter and spring, but are contrary to those of Taylor *et al.* (1985) who reported that sperm production in Holstein bulls (ejaculate volume, sperm concentration and total sperm number) was greater during the summer in temperate environments. Moreover, Teixeira *et al.* (2011) reported that for fresh semen, the ejaculate in April had less volume and sperm concentration, while with the frozen/thawed semen, the proportion of sperm was greater in April to July, decreasing from October to December. Thus, they suggested that reproduction with natural mating should be successful at any time of the year, but the best time of year for using frozen semen is from June to September. Seasonal effects may be due to various factors such as temperature, humidity, photoperiod, feed composition and management. The decrease in sperm production of Holstein bulls housed in Morocco during summer season may be explained by the high temperature recorded during this season. However, Everett & Bean (1982) and Brito *et al.* (2002) did not find any significant effects of ambient temperature or humidity on sperm production and semen quality. Likewise, Taylor *et al.* (1985) demonstrated that extreme temperatures (–24 to –19 °C and 27 to 32 °C) had only minor detrimental effects on semen production.

Year of collection

Semen traits were significantly influenced by the year of collection ($P<0.001$). Ejaculate volume has increased over years; but no clear trend was observed for the other semen characteristics. These results are in accordance with those of Karoui *et al.* (2011). The significant differences in semen production between years may be due to changes that are usually observed in e.g. feed quantity and quality, climatic conditions, management practice, equipment, freezing protocol or collectors and technicians.

Interval between collections

The number of days between two collections affected all studied traits ($P<0.001$) (Table 4). Large effects of collection interval were also found by Everett & Bean (1982), Mathevon *et al.* (1998), Fuerst-Waltl *et al.* (2006) and Karoui *et al.* (2011). Increasing collection intervals resulted in significant higher semen characteristics. However, when the length between two collections was four days, ejaculate volume and motility declined slightly, whereas sperm concentration and total number of spermatozoa continued to increase.

Table 4
Number of observations, least-squares means±standard errors of semen production and semen quality of Holstein bulls[1]

Factor	Number	Ejaculate volume (ml)	Sperm concentration (109 x ml-1)	Number of spermatozoa per ejaculate (109)	Mass motility score	Individual motility (%)	Post-thawing motility (%)
Interval between collections (days)		***	***	***	***	***	***
1	25	4.67±0.33^{a}	0.91±0.03^{ab}	4.38±0.38^{ab}	3.35±0.10^{ab}	74.9±1.11^{abc}	58.9±1.09^{ab}
2	1 883	4.76±0.20^{b}	0.93±0.01^{a}	4.46±0.22^{a}	3.48±0.04^{a}	75.8±0.53^{ac}	59.1±0.58^{a}
3	682	4.90±0.20^{c}	0.92±0.01^{a}	4.57±0.23^{a}	3.50±0.04^{ab}	75.9±0.56^{ac}	59.3±0.61^{a}
4	187	4.84±0.22^{c}	0.94±0.02^{a}	4.58±0.25^{a}	3.45±0.05^{ab}	74.1±0.64^{b}	58.1±0.67^{b}
5	741	5.11±0.20^{c}	0.96±0.01^{b}	4.96±0.22^{b}	3.55±0.04^{b}	76.2±0.55^{a}	58.6±0.60^{ab}
≥6	528	5.43±0.20^{d}	0.93±0.01^{ab}	5.16±0.23^{b}	3.43±0.04^{a}	75.3±0.56^{bc}	58.2±0.61^{a}
Ejaculate order		***	***	***	***	***	ns
1st	2 460	5.38±0.20^{a}	0.99±0.01^{a}	5.36±0.22^{a}	3.61±0.04^{a}	76.6±0.55^{a}	58.6±0.60
2nd	1 586	4.53±0.20^{b}	0.88±0.01^{b}	4.01±0.23^{b}	3.31±0.04^{b}	74.2±0.56^{b}	58.8±0.61

[1]Least-squares means within a column and a treatment that do not have a common superscript (a-d) are significantly different (P<0.05). ns: not significant; P>0.05, ***P<0.001

Table 5
Number of observations, least-squares means (LSM) ± standard errors (SE) for number of motile spermatozoa per month, total number of motile spermatozoa per month and estimated number of AI doses produced per month of Holstein bulls according to the interval between collections and the ejaculate order[1]

Interval between collections, days	Number of motile spermatozoa per month[2] (×109) First ejaculate		Second ejaculate		Total number of motile (x109) spermatozoa per month[3]	Number of AI doses produced per month[4]
	Number	LSM±SE	Number	LSM±SE		
1	15	70.4±4.00^{a}	10	53.9±4.73^{ab}	124.3	5 179
2	1 164	54.2±1.85^{b}	719	37.8±1.88^{c}	92.0	3 833
3	443	37.8±1.93^{c}	239	24.5±2.03^{def}	62.3	2 596
4	112	27.5±2.24^{d}	75	18.8±2.42^{ef}	46.3	1 929
5	417	24.9±1.93^{d}	324	20.9±1.97^{f}	45.8	1 908
6	309	24.7±1.97^{de}	219	22.4±2.05^{df}	47.1	1 962

[1]Least-squares means within both columns that do not have a common superscript (a-h) are significantly different (P<0.05). [2]Number of motile spermatozoa per month= ejaculate volume × sperm concentration × individual motility × number of collections per month allowed by a given interval between collections. [3]Total number of motile spermatozoa per month is the sum of number of motile spermatozoa per month obtained at first and second ejaculates. [4]Each AI dose contained 24 million spermatozoa.

Ejaculate order

Differences between first and second ejaculates were significant for all studied semen traits (*P*<0.01 to *P*<0.001), except for post-thawing motility (*P*>0.05). In general, higher semen quantity and quality was observed for first ejaculates. Differences were 0.85 ml, 0.11×109 per ml, 1.35×109 spermatozoa, 2.4 % and 0.30 for volume, sperm concentration, total number of spermatozoa per ejaculate, individual motility and mass motility score, respectively. Ejaculate volume, sperm concentration and hence total number of spermatozoa per ejaculate were the characteristics most affected by ejaculate order, since first ejaculates were 18.8 %, 12.5 % and 33.7 % higher than second ejaculates. Similarly, several researchers (Everett & Bean 1982, Taylor *et al.* 1985, Fuerst-Waltl *et al.* 2006, Karoui *et al.* 2011) reported that second ejaculates were inferior in sperm quantity and quality, but not with the same importance for all traits. Differences due to collection order may be explained by differences in intensities of sexual preparation and stimulation of bulls prior to each ejaculate.

To answer to the question asked by the centre's manager concerning the optimal interval between two collections for a maximum number of straws, the number of motile spermatozoa produced per month was calculated (ejaculate volume × sperm concentration × individual motility × number of collections per month allowed by a given interval between collections). It was assumed that when the interval between two collections was 1, 2, 3, 4, 5 and 6 days; the numbers of collections allowed within five working days per week were 22, 13, 9, 7, 5 and 5 collections during a month period, respectively. Thus, the analysis of number of motile spermatozoa produced per month, by adding the interval between two collections × ejaculate order interaction to the mixed model used previously, showed that bulls collected once a day at one day interval produced per month 30 %, 86 %, 156 %, 183 % and 185 % more motile spermatozoa than those collected once a day at 2, 3, 4, 5 and 6 days interval, respectively (Table 5). Bulls' sperm collected twice a day (sum of motile spermatozoa obtained at 1st and 2nd collections) at 1, 2, 3, 4, 5 and 6 days interval produced per month 77 %, 70 %, 65 %, 68 %, 84 % and 91 % more than those collected once a day at the same interval, respectively. The current results support a short interval between collections and two collections per day for maximising sperm production. These findings are in agreement with those of Mathevon *et al.* (1998).

Repeatability estimates

Estimates of repeatability for semen traits of Holstein bulls were medium to high. They varied from 0.157 for mass motility score to 0.411 for ejaculate volume (Table 6). The higher repeatability estimates indicate that bulls tend to have the same performance for traits with high estimates. Taylor *et al.* (1985) reported that repeatability estimates for volume, concentration and number of spermatozoa per ejaculate were 0.26, 0.23 and 0.37, respectively. In addition, Mathevon *et al.* (1998) found that repeatability estimates varied with age of bulls. They were higher for mature bulls (from 0.51 to 0.64) than for young bulls (0.41 to 0.53) owing to their more stabilized semen production.

In conclusion, this study showed that semen production and sperm quality of Holstein bulls housed in Moroccan conditions were similar to those of bulls raised in temperate conditions. Better adaptation of Holstein bulls to environmental conditions could have been responsible for this conclusion. Also, semen characteristics were mostly affected by the environmental factors. Volume and concentration were more affected by age, season and ejaculate order. This study allowed the confirmation that the highest number of AI doses were obtained from short intervals between collections and a high number of collections per day.

Table 6
Variance components and repeatability estimates for semen traits of Holstein bulls

Semen traits	σ^2_{bull}	σ^2_e	Repeatability estimates
Ejaculate volume	1.204	1.724	0.411
Sperm concentration	0.004	0.021	0.175
Number of spermatozoa per ejaculate	1.497	2.334	0.391
Mass motility score	0.037	0.199	0.157
Individual motility	8.202	23.754	0.257
Post-thawing motility	10.368	21.197	0.328

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