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Mass sperm motility is associated with fertility in sheep

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ABSTRACT

The study was to focus on the relationship between wave motion (mass sperm motility, measured by a mass sperm motility score, manually assessed by artificial insemination (AI) center operators) and fertility in male sheep. A dataset of 711,562 artificial inseminations performed in seven breeds by five French AI centers during the 2001–2005 time period was used for the analysis. Factors influencing the outcome of the insemination, which is a binary response observed at lambing of either success (1) or failure (0), were studied using a joint model within each breed and AI center (eight separate analyses). The joint model is a multivariate model where all information related to the female, the male and the insemination process were included to improve the estimation of the factor effects. Results were consistent for all analyses. The male factors affecting AI results were the age of the ram and the mass motility. After correction for the other factors of variation, the lambing rate increased *quasi* linearly from three to more than ten points with the mass sperm motility score depending on the breed and the AI center. The consistency of the relationship for all breeds indicated that mass sperm motility is predictive of the fertility resulting when sperm are used from a specific ejaculate. Nonetheless, predictability could be improved if an objective measurement of mass sperm motility were available as a substitute for the subjective scoring currently in use in AI centers.

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1. Introduction

Artificial insemination (AI) in the French sheep farming industry dates back to the early 1970s. It is mainly performed by cervical insemination of fresh semen on estrous synchronized females. The success of AI depends on factors

related to male and female fertility as well as factors related to the estrous synchronization and insemination practices (David et al., 2008). Because a single ejaculate is used to perform several inseminations, it is important to put forward criteria which permit a successful selection of fertile sperm to be used for dose production. Many relationships among sperm characteristics and fertility have been considered in previous studies with various species. A sperm that participated in the fertilization process should be able to rapidly transit the female reproductive tract to the oviducal region, penetrate the outer membranes of the oocyte

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Table 1

Fertility after AI of several sheep breeds.

Breed	Period of record	Number of AI	Number of rams	Number of ewes	Fertility (observed lambing rate)
Lacaune 1 (LAC1)	2001–2005	247 651	1 433	123 574	66.7
Lacaune 2 (LAC2)	2001–2005	227 633	1 517	117 384	65.8
Manech tête rousse (MTR)	2001–2005	140 722	963	77 422	56.8
Basco-Béarnais (BaB)	2001–2005	34 579	257	18 947	55.6
Mouton vendéen (VEN)	2002–2005	6 049	83	4 488	54.7
Manech tête noire (MTN)	2001–2005	32 793	220	17 295	54.6
Blanc du massif central (BMC)	2004–2005	15 863	108	13 061	48.7
Texel (TEX)	2004–2005	6 272	59	4 964	48.2

(which necessitates acrosomal and cytoplasmic membrane integrity) and contribute to formation of an embryo (for which nuclear integrity is required). Different methods, consisting of functional and non-functional assessments of sperm, have been proposed to evaluate the various aforementioned characteristics of the sperm cell. The functional assessments are the: (1) Cervical mucus penetration test which is used to assess the number of sperm retained in the oviduct; (2) Penetration test of the zona pellucida, relationship between this test and fertilizing capacity of sperm is still controversial (Larsson and Rodriguez-Martinez, 2000; Rodriguez-Martinez, 2003); and (3) *In-vitro* fertilization test, conducted *a priori* and is the most relevant test to evaluate sperm fertilization capacity (Gadea, 2005). Nevertheless, the correlation between this test and *in vivo* fertility results has yet to be convincing with inconsistent results in bulls (Zhang et al., 1999; Rodriguez-Martinez, 2003). Non-functional tests are the: (1) Measurement of seminal proteins and (2) Chromatin integrity test. In some studies, results obtained from using this latter test are correlated with male fertility (Evenson and Jost, 2000; Januskauskas et al., 2003). Other non-functional tests are the: (3) Plasma membrane status and (4) Acrosome tests the latter of which has been found to not provide any advantage compared with the more conventional tests in pigs (Gadea, 2005) or the: (5) Percentage of abnormal/dead sperm test which is related to fertility in many species (Linford et al., 1976; Correa et al., 1997; Rodriguez-Martinez, 2003; Malo et al., 2004; Gadea, 2005). Assessments related to the determination of sperm movement can be indirect estimates of motion using (6) ATP (Adenosine Triphosphate) measurements or directly by the observation of groups of sperm ((7) Mass sperm motility) or use of the individual cell motion test (Computer Assisted Sperm Analysis: (8) CASA; (Boyer et al., 1989; Amann and Katz, 2004). The correlation between various tests is variable (Gadea, 2005) which is not surprising because tests do not assess the same variables. This is likely to be the reason that it is recommended that a combination of tests be used to provide a more reliable estimate of fertilizing capacity of sperm (Rodriguez-Martinez, 2003). Performing numerous tests is, however, not practical for sheep AI centers to conduct because the processes are too expensive and lengthy. For years, French AI sheep centers have selected ejaculates for insemination based on mass sperm motility score. This rapid test has the advantage of being easy to perform, inexpensive from an

economic perspective and predictive of sperm fertilizing capacity (David et al., 2008). Nonetheless, other predictive criteria of sperm fertility are currently being investigated (Nordstoga et al., 2013; Vicente-Fiel et al., 2014). Using reproduction data from many French sheep breeds, the present study aimed to reassess the relationship between mass sperm motility and male fertility.

2. Materials and methods

2.1. AI centers and sheep breeds

This study was based on a total of 711,562 AI performed during the 2001–2005 time period. The semen used for AI was produced by rams belonging to seven breeds and located in five French AI centers (Table 1). These seven breeds include four dairy breeds: Manech Tête Rousse (MTR), Manech tête noire (MTN), Basco Béarnaise (BaB), and Lacaune (LAC) and three meat breeds: Texel (TEX), Mouton Vendéen (VEN), and Blanc du Massif Central (BMC). The rams of the dairy breeds were located in three AI centers. Rams of the MTR, MTN and BaB breeds were housed in one center and the LAC rams were housed in two other AI centers (identified as LAC1 and LAC2 rams). The meat breed rams were located in two AI centers. The TEX and VEN rams were housing in one center and BMC rams in another AI center. To synchronize the increase of semen production with the desired insemination period, rams received a melatonin implant (Mélovine® (CEVA, Santé animale, Libourne, France), MTR, MTN, BaB, BMC) or a photoperiodic treatment (LAC, TEX, VEN) about 2 months before the beginning of the annual semen collection period at the centers (Chemineau et al., 1988).

2.2. Semen collection and motility assessment

Ejaculates were obtained after natural ejaculation using an artificial vagina. Semen collection consisted of a pool of one to three successive ejaculates of a given ram, obtained over a 2–5 min period. Semen volume, sperm concentration, and mass motility of each pool were assessed immediately after collection. Volume was read from measuring the collection inside a glass tube. At a dilution of 1:400 in 0.9% sodium chloride solution, the sperm concentration was assessed using a standard pre-calibrated spectrophotometer (Evans and Maxwell, 1987). A drop of

Table 2

Mass motility rating system for ejaculated ram sperm.

Rating	Microscopic appearance ^a
0	No swirl – nil or sporadic oscillation of individual sperm
1	No swirl – generalized oscillation of individual sperm only
2	Very slow distinct swirl
3	Slow distinct swirl
4	Moderately fast distinct swirl
5	Fast distinct swirl

^a Drop of 5 μ L of raw semen deposited on a pre-warmed glass slide ($\approx 37^\circ\text{C}$). Edge of the drop is observed at low magnification ($10\times$ objective) on the thermally controlled stage of a phase contrast microscope.

5 μ L of raw semen was deposited on a pre-warmed glass slide ($\approx 37^\circ\text{C}$) and the edge of the drop was observed at low magnification ($10\times$ objective) on the thermally controlled stage of a phase contrast microscope. Observations at the edges of the drop provide for assessment of the rapid flogging of black waves and whirlpools on a grey background which is termed as the wave motion or mass sperm motility. This mass sperm motility was scored subjectively from 0 (no motion) to 5 (numerous rapid waves) on a scale with steps equal to 1 according to the original method described by Evans and Maxwell (1987) (Table 2). Observations of the ram sperm video with the mass sperm motility score can be found in supplementary content of the present manuscript. Given the high-quality of ejaculates produced by AI rams, most of the scores were between 4 and 5. Therefore, the wave motion scoring was refined with 0.1 steps between 4 and 5 based on the rotation speed of the waves to more precisely describe the variability between ejaculates. This refined scoring is performed on the basis of the experience and knowledge of the technicians and as a result of competition among AI centers, no information about the criteria used to perform the refined scoring is available. Within an AI center, the same team of operators assessed the mass motility during the entire period of study. Because of the scoring subjectivity, each team had its own score that could slightly differ from the standard 0 to 5 of the continuous scale and from the generalized scoring system between 4 and 5. There was no sperm motility score greater than 4.5 for the LAC2 breed and a 0.25 step between scores 4 and 5 for the VEN and TEX breeds was used. Only ejaculates with a wave motion score of greater than 4 (4.5 for the BMC breed) were kept for AI, which corresponds to more than 80% of the ejaculates that were collected. Selected semen was then diluted in a skim milk extender (11.1 g/100 mL of water) supplemented with antibiotics at a final concentration ranging from 1.0 to 1.6×10^9 sperm/mL depending on the breed and the AI center. Diluted semen was packaged in 0.25 mL straws and stored at 15°C until cervical insemination was performed within 6 h following collection. Before insemination, ewes received an estrous synchronization treatment (Fluorogestone acetate vaginal sponge (Sanofi animal health Ltd, France or Intervet, Beaucauze, France) inserted for 14 days, and a Pregnant Mare Serum Gonadotropin injection at withdrawal (Folligon® or PMSG; Sanofi animal health Ltd, France)). Insemination was performed 55 h following sponge removal without detection of estrus. To enhance pregnancy rates, ewes were joined with entire males 6 days after AI.

2.3. Analysis of fertility data

AI was defined as a success ($y = 1$) if lambing occurred during a breed specific appropriate interval of time after AI, otherwise it was considered as a failure ($y = 0$). The intervals of time after AI were 141–151 d for the VEN breed, 142–152 d for the LAC and TEX breeds, 143–153 d for the BMC breed, 144–156 d for the BaB breed, and 144–158 d for the MTR and MTN breeds. The AI result ($y = 0$ or 1) was the variable of interest because the mean for this variable corresponded to the lambing rate. For a given insemination, information was collected from the AI center that made the semen collection and recorded the insemination data and from the French national performance recordings through which data are assimilated for each individual ewe's production performance. Thus, a detailed description of each insemination (from semen collection and female estrous synchronization to lambing) was available. It was then possible to study how the lambing rate was affected by factors related to females (estrous synchronization, reproductive, and production), males (sperm characteristics, collection procedures), insemination procedures (AI operator, interval between collection and AI) or by factors common to all of the previous categories (year, season, herd).

Separate analyses within breed/center were performed. Linear mixed models were used to select the factors influencing AI success. All fixed effects and one-way interactions of biological relevance included in the models were selected in a step-wise manner, using nested models that were compared with each other with the likelihood ratio test. Random effects were selected using the restricted likelihood ratio test. The distribution of this statistical test under the null hypothesis of variance equal to 0 is a 50:50 mixture of χ^2_q and χ^2_{q+1} distribution (Morrell, 1998) where q is the number of random effects in the reduced model (residual effect excluded). The list of the tested environmental factors is presented in Table 3 (detailed information can be found in (David, 2008)). Once the final model was chosen for each breed/center, generalized linear mixed models (logit link function) were used to estimate the effect of mass sperm motility on the AI result adjusted for all the other significant factors of variation.

3. Results and discussion

For years, physiologists, biologists and geneticists aimed at improving fertility. A reduced fertility has important negative consequences. In animal production, a decrease in fertility results in a reduction in the number of offspring as well as diminishing the progress made in genetic selection. In human, it induces stress and depression (and other psychological disorders) (Hart, 2002). Consequently, many studies have been performed to identify the factors that are related to fertility. Such studies are not easy to perform because the reproduction outcome is a complex trait with both sexes of the species having many physiological and behavioral processes that impact success of reproduction. It is, therefore, difficult to identify the relationships among the many factors that contribute to fertility of individual animals. Tomlinson et al. (2013) suggest that the

Table 3

List of factors tested in the models to study the relationships between mass sperm motility and fertility for seven breeds of sheep.

Factors related to female fertility	Factors related to male fertility	Factors related to AI process	Common factors
Age of female (in years)	Age of male (in years)	Set of AI within flock-year	Year \times fortnight
Number of previous lambing	Interval between semen collections (in days)	Interval time between set of AI (in weeks)	Flock \times year (AI operator)
Age at first lambing (in months)	Number of ejaculate at each collection	Number of AI per operator within a set of AI (class of 50)	
Lambing – AI interval	Collection period (AM–PM)	Time interval between end of female treatment – AI (in hours)	
Type of previous reproductive event (AI success/failure, natural mating success/failure)	Initial semen concentration (in class)	PMSG dose (4 classes)	
Litter size at the previous lambing	Mass motility (in class)	Time interval between semen collection and AI (in hours)	
Total number of treatment	Semen dilution (straw/initial concentration)	AI operator	
Class of milk yield (4 quartiles within flock \times year) ^a			
Milking status (dry, in lactation, unknown) ^a			

Factors in italic were included as random effect.

^a For milk breed only (LAC, MTR, MTN, BB).

difficulty in explaining inconsistent results from different experiments is contributed to by the small numbers of animals used in many of the studies and recommends that research be conducted with large populations where both male and female factors are taken into account in the analysis. The only species where the causes of infertility are well documented is with humans after natural mating (Forti and Krausz, 1998). The authors reported that infertility was due to a female factor in 35% and a male factor in 30% of the cases as well as to abnormalities detected in both partners in 20% of the cases whilst for the remaining 15% of the cases there was no diagnosis that could be made. In animals, David et al. (2009) have recently proposed a model that can identify which gender is at the cause of infertility after artificial insemination. Findings depended on the species as well as the way AI was performed (David et al., 2011).

In the present study, data resulting from the French national performance recordings and AI centers were used. Information from both sexes was used for analysis of the large data set which allows for a strong statistical relevance of the results. Furthermore, fertility was assessed from *in vivo* results which are more reliable fertility indicators than *in vitro* findings (Rodriguez-Martinez, 2003). In the context of animal AI, a single ejaculate is used to inseminate several females. Thus, being able to select the ejaculates that will be used to produce the straws of semen used for insemination is a key component in a breeding selection scheme (Colenbrander et al., 2003). This is why, in the present study, the relationship between male factors, especially mass sperm motility, and fertility were the focus while other factors of variation were used as correction variables but were not of interest as related to the primary goal of the study.

The variation of the overall lambing rate with mass sperm motility score is presented in Fig. 1. For all breeds/centers, the same general trend was observed regardless of the average lambing rate, namely an increase

of the lambing rate with a corresponding increase in mass sperm motility score. After selection, the factors significantly related to lambing rate (alpha risk = 5%) were consistent between breeds/centers. The main factor was the year \times fortnight combination which is an uncontrollable factor. The two most important factors related to female fertility were the type of reproductive event the previous year and the time interval between previous lambing and AI. The significant factors related to male fertility were the age of the ram, dilution of the semen and mass sperm motility. For all breeds/centers, after correction for the other factors of variation, the lambing rate increased *quasi* linearly with the mass sperm motility score. For the MTR, BaB, and VEN breeds, this increase was more than ten points between extreme mass motility classes. For the other breeds the increase was about six points except for the LAC2 breed where it was three points. Fig. 2 depicts the variation of the lambing rate with mass sperm motility adjusted for the other factors of variation (LSMeans) for three very different breeds: LAC1, MTR, and BMC. Correction for the other factors of variation improved the relationship between mass sperm motility and lambing rate in comparison with the variation of overall lambing rate with mass sperm motility. Nonetheless, it was noted that the relationship was not linear over the entire range of motility scores. The slope of the curve was negative between the two lowest motility scores for the LAC1 and BMC breed and nearly null for MTR. This result shows that AI center technicians have some difficulties in scoring mass sperm cell motility in cases where there is little mass sperm motility (in the 4–5 range). By extrapolation, it can be postulated that some ejaculates with adequate capacity for fertilization have been discarded (not used to produce doses) because the mass sperm cell motility was inappropriately scored as less than 4 and vice versa some ejaculates with poor capacity for fertilization were retained for AI purposes. Performing a similar analysis on data where ejaculates have not been selected for AI could confirm this

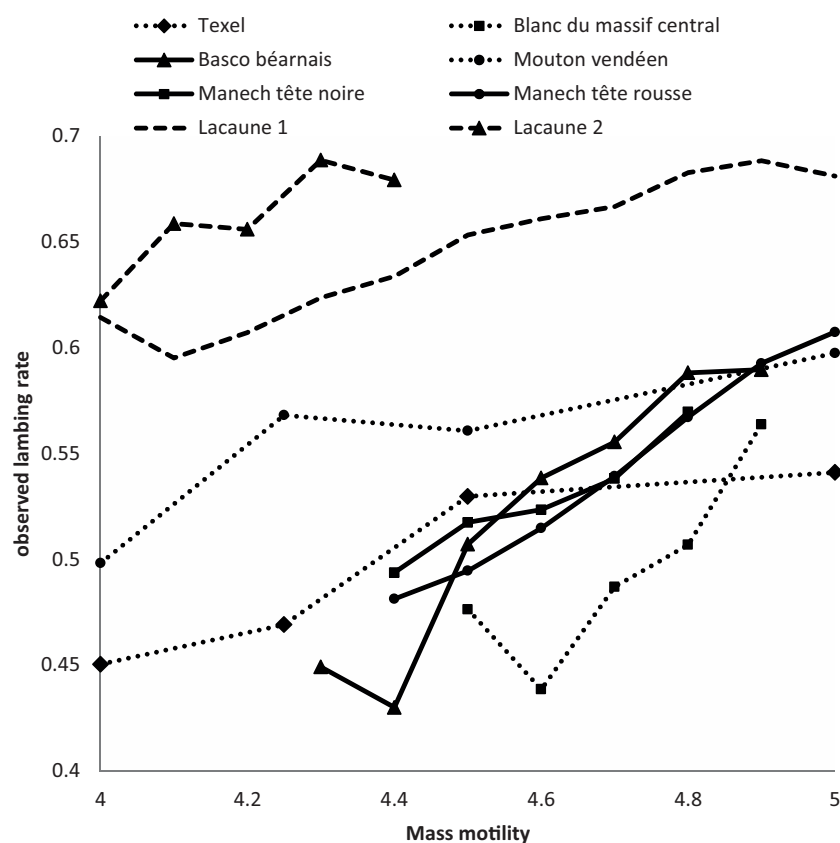


Fig. 1. Variations in observed lambing rate (number of lambing/number of AI) with mass sperm motility score (4–5) for seven breeds of sheep.

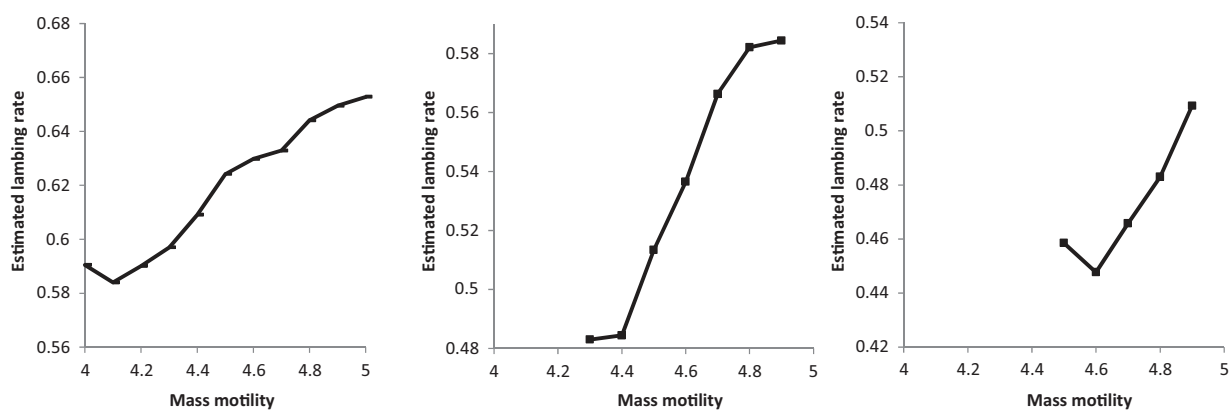


Fig. 2. Variations in estimated lambing rate (number of lambing/number of AI) with mass sperm motility score (4–5) for Lacaune (left), Manech Tête Rousse (middle), and Blanc du Massif Central (right) breeds.

hypothesis but no AI center has the desire to take the risk of implementing such a practice. A large number of studies have evaluated the relationship between mass sperm motility and overall fertility in different species. The results vary from no association (Colas, 1981; Duval et al., 1995; Zhang et al., 1999; Malo et al., 2004) to a positive correlation (Linford et al., 1976; Correa et al., 1997; Colenbrander et al., 2003; Foote, 2003; Theau-Clément et al., 2011) that is not species specific. The reported variability probably results from different experimental conditions as well as from the

subjectivity of mass sperm motility scoring (Rodriguez-Martinez, 2003). In the present study, there was agreement between results obtained within different breeds/centers. It is believed this is because the same sperm sampling and preparation methods were used in all AI centers. Although working in different AI centers, nearly all the technicians had received the same training to evaluate mass sperm motility.

The use of CASA that allows a detailed quantitative measurement of individual sperm cell motility should provide

more reliable results than mass sperm motility to predict fertilizing capacity of semen doses (Vincent et al., 2014). However, studies linking CASA parameters to fertility have not clearly demonstrated a greater predictive fertility capability for bulls (Kjaestad et al., 1993; Farrell et al., 1998; Januskauskas et al., 1999; Gillan et al., 2008). The CASA parameters and mass sperm motility provide different information on the movement of sperm. The CASA analysis utilizes parameters from the 2D motion of individual sperm. Consequently, some information on sperm fertility potential are not considered with CASA analysis, in comparison with mass sperm motility where the 3D collective motion that is observed. Furthermore, mass sperm motility takes into consideration the collective movement of sperm. From taking into account cooperative effects of cells, different information is obtained as compared with the average individual motility provided by CASA. The strong relationship between mass sperm motility and fertility and the consistency of the results between breeds obtained in the present study provide evidence for this test for sperm selection to be used in AI. Furthermore this test is easy to conduct and rapid to perform and thus can be used routinely. Nonetheless, this test suffers from a major drawback: its subjectivity. The differences observed in the present study between LAC1 and LAC2 groups in the estimated increase of the lambing rate between extreme mass sperm motility classes (7 compared with 3 points in increase of lambing rate) illustrate this problem. The LAC1 and LAC2 groups correspond to the same breed, located in the same area of France where insemination is performed similarly and there is a similar average lambing rate with the only difference being the AI team. To avoid such variations in assessing the mass sperm motility score, it should be necessary to develop an objective assessment of the mass sperm motility similar that currently available for individual sperm cell motility. There are several ongoing studies on this subject. Mathematical models used for assessing the movement of the waves resulting from mass sperm motility are being developed (Degond et al., 2015; Degond and Yu, 2015). The parameters of these models can be used to provide a greater objective measurement of mass sperm motility. The relationship among these parameters and fertility has to subsequently be analyzed to ascertain whether this methodology provides an advantage over subjective scoring of mass sperm motility.

4. Conclusion and perspectives

Results obtained in the present study indicate mass sperm motility is a convincing indicator of fertility in sheep. It has the advantage of being inexpensive from a fiscal perspective with easy to conduct methodologies and is rapid to perform but it suffers from the drawback of being a subjective assessment. The development of an objective measurement of mass sperm motility is currently under way. If effective and efficient methods are developed, this should reduce the inappropriate scoring of mass sperm motility in ejaculates and the discarding of samples that could be effectively used for AI that with present methods have poor mass sperm motility scores. This will allow for an increase in the number of sperm doses produced per

day per AI center and thus enhance the efficiency of the center. For practical use in sheep AI centers that use fresh semen, the evaluation method that is being developed must be rapid. However, if development of rapid methodologies cannot occur the newly developed method can be used for frozen semen provided that there is a positive correlation between objective mass sperm motility and fertility established.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.anireprosci.2015.08.006>.

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