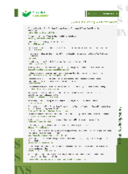
JURNAL SOSAINS

JURNAL SOSIAL DAN SAINS

VOLUME 4 NOMOR 5 2024
P-ISSN 2774-7018, E-ISSN 2774-700X



Improvement of the Quality of Landrace Pig Spermatozoa in Diluent of Young Coconut Water and Tris Combination

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Keywords:
Young coconut water, tris, spermatozoa, landrace boars.

ABSTRACT

Background: *The research material used was fresh semen of 2-year-old landrace boars of good quality (spermatozoa motility ≥ 70% and spermatozoa abnormality < 20%)*

Purpose: *This study aimed to find the best combination of young coconut water (YCW) and Tris to improve the quality of landrace boars spermatozoa.*

Method: *%). This study used an experimental method according to a complete randomized design procedure with five treatments and five replicates. The treatments were: T0= YCW, T1= TCW 75% + tris 25%, T2= YCW 50% + tris 50%, T3= YCW 25% + tris 75%, T4= Tris. All treatment diluents were added with 20% egg yolk, 3% moringa leaf extract, 1,000 IU/mL penicillin, and 1,000 µg/mL streptomycin. The variables tested were motility, viability, abnormality, and survival.*

Results*: The observation results at the 40th hour of storage showed that treatment T3 was significantly different (P<0.05) on motility, viability, abnormality and survival but on the variable viability treatment T3 was insignificantly different from T1 (p>0.05), but significantly different from treatment T0, T2, and T4; and for spermatozoa abnormality variables in all treatments were the same (p>0.05)*

Conclusion*: It was concluded that the combination of YCW 75% and tris 25% and 100% tris effectively maintained the quality of landrace boars spermatozoa.*

INTRODUCTION

Porcine semen is *voluminous,* has ejaculation with a volume of about 100 – 500 ml and a low concentration of spermatozoa of 200-300 x 106 cells/ml (Butta, Gaina, & Foeh, 2021). Pig semen has a fairly high level of unsaturated fatty acids in the phospholipids of the plasma membrane of spermatozoa. The use of fresh cement for a long period of time requires preservation with the addition of diluent materials that contain sufficient energy and nutrients sources, *buffer materials*, cold shock resistant materials, able to provide protection against bacterial contamination, and can protect spermatozoa during the processing and storage process (Rizal & Thahir, 2016).

The addition of thinners aims to meet nutritional needs and provide a suitable environment for spermatozoa. In addition, the thinner must also be able to protect

spermatozoa from damage due to temperature, and be made from inexpensive materials and their availability is easy to obtain (Muhammad, 2018). According to previous research, the process of dilution of cement can cause a decrease in the concentration of substances contained in the cement plasma such as amino acid levels, and ions that can change the balance of osmose pressure in the diluent which can affect motility and vitality (Nugroho & Nur, 2018).

Coconut water can be used as an alternative diluent that is easy to get because it is widely available in the surrounding environment and is affordable. Some simple carbohydrates, minerals and other substances in the diluent required by spermatozoa can be fulfilled from coconut water. This is in accordance with the opinion of previous researchers that coconut water is an isotonic liquid that is easy to obtain and contains carbon elements such as simple carbohydrates, glucose, sucrose, and fructose (Kholvi, 2019).

The use of coconut water as a cement diluent cannot be used alone but must be combined with other materials that contain buffer elements to maintain a pH that is in accordance with the needs of spermatozoa.

Tris is a solution containing citric acid that has a role as a buffer to prevent pH changes due to the buildup of lactic acid derived from the residual results of spermatozoa metabolism (Kusumawati, 2022). In addition, tris also contains fructose which can be used by spermatozoa as a source of energy (Hoesni, 2016). Tris serve as a buffer to prevent pH changes. Previous research has shown that tris aminomethane functions as a good buffer with low toxicity in high consistency (Faizal, 2016).

The combination of AKM and tris at the right level is expected to produce a diluent that is conducive to supporting the motility and viability of landrace pig spermatozoa during *in vitro* storage. This study aims to find the best combination of Young Coconut Water (AKM) and tris to improve the quality of landrace pig spermatozoa, while the benefits of this research are to improve the quality of Landrace pig semen, develop cheaper and more readily available semen thinner alternatives, and improve animal welfare.

RESEARCH METHODS

This study uses a laboratory experiment method with a basic design of a complete random design consisting of five treatments and five replicates. The treatments used in this study are as follows: P0 = AKM 100% P1 = AKM 75% + tris 25%, P2= AKM 50% + tris 50%, P3= AKM 25% + tris 75%, P4= tris 100%. The livestock used as a source of semen is a 2-year-old landrace male pig, which is in good health, has normal reproductive organs and has been trained to accommodate the semen. The research data was analyzed by *Analysis of variance* and continued with the Duncan test. The analysis was carried out with the help of SPSS 26 For windows software.

RESULTS AND DISCUSSION

Effect of Treatment on Spermatozoa Motility

Motility is the motility ability of spermatozoa (Azzahra, Setiatin, & Samsudewa, 2016). This progressive movement is used as a benchmark for spermatozoan cell fertility

because it is expected to be able to accelerate the meeting with the egg (ovum) for the fertilization process in the female reproductive tract (Mahfud, Isnaini, Yekti, Kuswati, & Susilawati, 2019). Motility values can be seen in Table 1.

Table 1. Effect of treatment on spermatozoa motility (%)

	Perlakuan %					
Jam ke-	**P0**	**P1**	**P2**	**P3**	**P4**	**P value**
0	80, 00 ± 3,53[a]	80, 00 ± 3,53[a]	80, 00 ± 3,53[a]	80, 00 ± 3,53[a]	80,00 ± 3,53[a]	1,000
8	64, 00 ± 2,23[b]	74,00 ± 2,23[a]	71,00 ± 4,18[a]	74,00 ± 2,23[a]	72,00 ± 2,73[a]	0,000
16	53,00 ± 2,73[b]	68,00 ± 2,73[a]	64,00 ± 6,51[a]	68,00 ± 2,73[a]	66,00 ± 2,23[a]	0,000
24	43,00 ± 2,73[c]	60,00 ± 3,53[ab]	55,00 ± 6,12[b]	62,00 ±4,47[a]	57,00 ± 5,70[ab]	0,000
32	33,00 ± 2,73[c]	51,00 ± 4,18[a]	44,00 ± 4,18[b]	54,00 ± 2,23[a]	50,00 ± 6,12[a]	0,000
40	23,00 ± 2,73[c]	41,00 ± 4,18[a]	33,00 ± 2,73[b]	43,00 ± 2,73[a]	40,00 ± 5,00[a]	0,000
48	12,00 ± 2,73[c]	31,00 ± 4,18[a]	24,00 ± 4,18[b]	34,00 ± 2,23[a]	30,00 ± 6,12[a]	0,000

Superscripts with different letters on the same line show a noticeable difference (P<0,05). P0= AKM, P1= AKM 75% + tris 25%, P2= AKM 50% + tris 50%, P3= AKM 25% + tris 75%, P4= tris.

The results of the analysis showed that the percentage of spermatozoa motility at 0 hour of storage did not show a difference between the treatments (p>0.05). This shows that all diluents still have conditions that are conducive to life and motility of spermatozoa, such as the pH of the diluent which is still at the optimal range, the availability of energy that is still abundant, and the content of other compounds that are still sufficient for the needs of spermatozoa. From the 8th to the 40th hour of storage, the rate of decrease in spermatozoa motility varied between treatments, so that at the 40th hour of storage, the combination treatment of young coconut water and tris (P1, P2, and P3) resulted in higher spermatozoa motility than coconut water diluent alone (P0) (p<0.05); however, the treatment of P1 and P3 was not significantly different from the treatment of P4 (Tris) (P>0.05). This indicates that the use of coconut water without being combined with tris is not optimal in maintaining the motility of landrace pig spermatozoa.

The high percentage of spermatozoa motility in the P1, P2, and P3 treatments can be caused by the presence of higher nutrient content compared to those contained in the P0 treatment. The nutrient content contained in the three treatments in addition to coming from glucose and fructose contained in the AKM is also supplied by the fructose contained in the tris diluent but the tris acts as a buffer material, maintains osmotic pressure, electrolyte balance and protects spermatozoa during the storage period, while in the P0 treatment, the energy that can be used by spermatozoa only comes from glucose and fructose contained in the AKM (Ramandhani, Agustini, & Suharto, 2022).

Fructose as a diluent will provide nutrients as an energy source in the form of ATP for spermatozoa so that it can last longer and young coconut water as a diluent has glucose and fructose content, so it is thought to be able to meet the nutritional needs of spermatozoa during storage (Riyadhi & Rizal, 2018). This should be known to get spermatozoa diluents that contain fructose so that they can increase motility and long life can also maintain pH.

In addition, in the treatment of P1, P2, P3 there are also buffer elements contributed by tris. The function of the *buffer* is to maintain the pH of the diluent so that it suits the needs of the sperm. A diluent that does not contain *a buffer* element can cause a more drastic decrease in the pH of the diluent due to lactic acid buildup as a byproduct of anaerobic spermatozoa metabolism.

Effect of Treatment on Spermatozoa Viability

The viability of spermatozoa is the vitality of spermatozoa in diluents, and is one of the variables that determine the quality of cement (Lestari, Ihsan, & Isnaini, 2014). Assessment of spermatozoa viability can be done objectively using differential staining. The average viability of spermatozoa in the treatment can be seen in Table 2.

Table 2. Effect of treatment on viability of spermatozoa (%)

	Treatment %					
Jam ke-	P0	P1	P2	P3	P4	P value
0	93,35 ± 4,09[a]	93,39 ± 4,47[a]	93,49 ± 4,33[a]	93,37 ± 4,16[a]	93,48 ± 4,24[a]	1,000
8	73,41 ± 2,39[d]	84,58 ± 2,08[ab]	81,14 ± 3,24[c]	86,17 ± 1,64[a]	82,76 ± 2,13[b]	0,000
16	62,36 ± 3,41[b]	81,39 ± 5,49[a]	74,40 ± 7,62[a]	82,32 ± 5,81[a]	78,78 ± 4,60[a]	0,000
24	51,73 ± 2,99[d]	71,29 ± 4,10[ab]	63,33 ± 5,58[c]	74,71 ± 4,95[a]	67,65 ± 6,00[b]	0,000
32	43,88 ± 1,46[d]	63,27 ± 5,11[ab]	52,88 ± 6,05[c]	66,37 ± 2,33[a]	59,01 ± 6,27[b]	0,000
40	34,35 ± 1,23[d]	50,16 ± 5,61[ab]	42,81 ± 3,95[c]	54,47 ± 2,93[a]	48,96 ± 4,32[b]	0,000
48	19,82 ± 5,04[c]	42,57 ± 5,97[a]	33,19 ± 7,90[b]	44,97 ± 3,22[a]	40,17, ± 6,50[ab]	0,000

Superscripts with different letters on the same line show a noticeable difference ($P<0,05$). P0= AKM, P1= AKM 75% + tris 25%, P2= AKM 50% + tris 50%, P3= AKM 25% + tris 75%, P4= tris.

Table 2 shows a decrease in the percentage of viability of spermatozoa in each treatment from the 8th to the 40th hour of storage. The decrease in the percentage of spermatozoa varies from treatment to treatment because the ability of each diluent is also different in maintaining spermatozoa survival.

The results of statistical analysis at 0 hour of storage showed that the treatment had no real effect ($P>0.05$) on the viability of spermatozoa; however, from the 8th and 16th hours, there was a significant difference between the treatment ($P<0.05$), with the highest viability produced by the P1 and P3 treatments. The decrease in spermatozoa viability in P0 treatment can be caused by the insufficient energy available for spermatozoa life, where the available energy only comes from coconut water so that when stored for a long period of time, the energy runs out quickly so that it is not enough to meet the needs of spermatozoa. In addition, in the P0 treatment, the pH drops quite drastically due to the accumulation of lactic acid which is the result of metabolic residues. The absence of buffer elements contained in the tris causes the diluent condition to quickly become acidic so that it is not conducive to the survival of spermatozoa. The decrease in motility, viability and integrity of the plasma membrane during the cooling process occurs due to temperature changes and extreme osmolality that damage the lipid composition of the plasma membrane which has an impact on decreasing the quality of spermatozoa (Sukmawati, Arifiantini, & Purwantara, 2014).

The higher percentage of viability of spermatozoa during the storage period is said to be very feasible for use in the artificial insemination process. This is suspected because it is influenced by the source of nutrients from egg yolks and young coconut water which contains protein, vitamins and fats as well as carbohydrates, fructose, glucose and sucrose which function as a food source for spermatozoa so that spermatozoa can survive for 0 hours to 40 hours of storage. AKM contains glucose and fructose which are also found in cement (pH ACIDITY, Berek, Dethan, & Tahuk, 2021). According to previous research, the storage of spermatozoa requires fructose which is used as an energy source for spermatozoa and AKM is a diluent that has fructose content (Faizal, 2016).

Effect of Treatment on Spermatozoa Abnormality

Spermatozoa abnormalities are physical abnormalities of spermatozoa that occur during the process of spermatozoa formation in the semiferial tubuli (primary abnormality) or during the passage of spermatozoa to the epdibranch, during storage in the epididymis, during ejaculation or during the processing and preservation of semen (secondary abnormalities). The average spermatozoa abnormalities in this study can be seen in Table 3.

Table 3. Effect of treatment on spermatozoa abnormalities (%)

Jam ke-	Treatment %					
	P0	**P1**	**P2**	**P3**	**P4**	**P value**
0	3,67 ± 0,60[a]	3,60 ± 0,45[a]	3,68 ± 0,56[a]	3,69 ± 0,48[a]	3,74 ± 0,62[a]	0,996
8	4,23 ± 0,71[a]	4,11 ± 0,70[a]	4,23 ± 0,72[a]	4,22 ± 0,72[a]	4,20 ± 0,79[a]	0,999
16	5,17 ± 0,24[a]	5,18 ± 0,13[a]	4,99 ± 0,61[a]	5,05 ± 0,56[a]	4,95 ± 0,69[a]	0,929
24	5,77 ± 0,51[a]	5,93 ± 0,47[a]	5,95 ± 0,36[a]	5,86 ± 0,73[a]	5,87 ± 0,38[a]	0,966
32	6,55 ± 0,63[a]	6,46 ± 0,64[a]	6,41 ± 0,75[a]	6,47 ± 0,59[a]	6,46 ± 0,62[a]	0,998
40	7,59 ± 0,63[a]	7,58 ± 0,54[a]	7,51 ± 0,63[a]	7,38 ± 0,76[a]	7,56 ± 0,66[a]	0,985
48	9, 40 ± 0,58[a]	9,44 ± 0,60[a]	9,29 ± 0,36[a]	9,19 ± 0,56[a]	9,41 ± 0,42[a]	0,932

Superscripts with the same letter on the same line show an intangible difference (P>0,05). P0= AKM, P1= AKM 75% + tris 25%, P2= AKM 50% + tris 50%, P3= AKM 25% + tris 75%, P4= tris.

Table 3 shows that up to the 40th hour of storage, the lowest percentage of spermatozoa abnormalities is found in the P3 treatment, and the highest is in P0, but statistically the five treatments have different spermatozoa abnormalities that are not significant ((P>0.05). The low abnormality in the P3 treatment is due to the content of two diluents with different doses that are able to prevent lipid peroxide and protect spermatozoa from cold *shock* during storage. The number of abnormal spermatozoa is increasing, which will lead to low fertility. The increase in abnormalities was also caused by the preparation of previous observations but caused by the presence of lipid peroxides (Yon Soepri, 2020). Fructose is not only an energy source but also to maintain the osmotic pressure of the diluent solution and maintain the integrity of the intact plasma membrane (Atmaja, Budiasa, & Bebas, 2014).

The percentage of spermatozoa abnormalities showed that from the beginning of observation to the end of the 40-hour observation there was an unreal difference (P>0.05) between treatments. This is because the administration of fructose at optimal levels is able to reduce the increase in abnormalities that occur due to lipid peroxidation at the same time. The abnormalities observed in this study are secondary abnormalities, which are characterized by a broken tail, a head separated from the tail, and a curved tail.

The storage process at the time of dilution also causes physical damage to spermatozoa. The percentage of abnormalities is still categorized as good as explained that the percentage of good spermatozoa abnormalities for artificial insemination is no more than 20%. Previous research has stated that the increase in abnormalities is caused by the lipid perioxidase process, changes in osmotic pressure due to free radicals and lactic acid resulting from metabolic processes, thereby damaging the plasma membrane and causing an increase in spetozoan abnormalities (Atmaja et al., 2014).

Effect of Treatment on Spermatozoa Survival

Survival is the ability of spermatozoa to maintain their survival for a certain time until motility decreases by up to 40 percent. The survival of spermatozoa can be seen in Table 4.

Table 4. Effect of treatment on spermatozoa survival (hours)

Treatment	Durability Life (Hours)
P0	26,40±2,19[c]
P1	40,80±3,34[a]
P2	34,66±2,49[b]
P3	42,40±2,19[a]
P4	39,46±5,04[a]
P value	0,000

Superscripts with different letters on the same column show a noticeable difference (P<0,05). P0= AKM, P1= AKM 75% + tris 25%, P2= AKM 50% + tris 50%, P3= AKM 25% + tris 75%, P4= tris.

The results of statistical analysis showed that the treatment was significantly different (P<0.05) for the survival of spermatozoa, with the highest survival produced by the P1, P3 and P4 treatments, and the three treatments were significantly different from the P0 and P2 treatments (P<0.05). AKM in the treatment of no *buffer* content and does not get additional energy supply from the fructose in the tris diluent causes AKM to produce low life endurance. Garner and Hafez (2000) stated that the fructose in the semen diluent is used by spermatozoa as an energy source both in anaerobic conditions (at the time of storage), and in aerobic conditions (in the female reproductive tract). The low survival of spermatozoa in treatment is caused by the presence of lactic acid residual cell metabolism which results in a decrease in pH and this condition can be toxic to spermatozoa which then results in low spermatozoa motility until death occurs. Excessive lactic acid in the diluent can cause a change in pH which can cause toxic effects and high mortality for spermatozoa.

As well as the administration of the right antioxidants provides maximum results to prevent lipid peroxide in the plasma membrane of spermatozoa by preventing or breaking the stability of the lipid peroxide chain in the plasma membrane of spermatozoa, so as to reduce the damage that occurs to the plasma membrane of spermatozoa. An intact membrane will cause the metabolic process to run properly, so that the energy produced is maximum. The water content of young coconuts can provide the physical and chemical needs of spermatozoa so that they can maintain the fertility and life endurance of spermatozoa.

In the condition that spermatozoa do not receive supplementation of nutrients and protective materials against cold shock as in the control treatment, spermatozoa will quickly die due to the depletion of energy substrates, because they only rely on materials

contained in the semen plasma and in spermatozoa cells, such as fructose and plasmalogen whose availability is very limited. In addition, the limited number of buffers in the semen plasma also contributes to the acceleration of spermatozoa death due to a decrease in pH due to lactic acid accumulation that occurs in an anaerobic respiration state. Another thing that also affects is the absence of protective elements in the semen plasma so that when spermatozoa are stored at low temperatures, there is damage to the cell membrane and results in death.

Spermatozoa of landrace pigs preserved in AKM and tris diluents have a longer survival than those of control or preserved in other treatments. The results showed that the survival of spermatozoa preserved in both diluents was significantly different ($P<0.05$) from the controls. This further reinforces the fact that diluents are so important in prolonging the life of spermatozoa *in vitro*. The depletion of the energy substrate that occurs during storage can cause the glycolysis process to produce energy to be unable to take place. In the absence of oxygen, the energy supply to spermatozoa is mainly donated through the glycolysis pathway.

CONCLUSION

It was concluded that the combination of AKM 75%, tris 25% and tris 100% was effective in maintaining the quality of spermatozoa of landrace pigs up to 40 hours of storage. Spermatozoa of landrace pigs preserved in AKM and tris diluents have a longer survival than those of control or preserved in other treatments. The results showed that the survival of spermatozoa preserved in both diluents was significantly different ($P<0.05$) from the controls. This further reinforces the fact that diluents are so important in prolonging the life of spermatozoa *in vitro.*

BIBLIOGRAPHY

Atmaja, Wahyu Kusuma, Budiasa, Made Kota, & Bebas, Wayan. (2014). Penambahan fruktosa mempertahankan motilitas dan daya hidup spermatozoa kalkun yang disimpan pada suhu 4oC. *Indonesia Medicus Veterinus*, *3*(4), 318–327.

Azzahra, F. Y., Setiatin, E. T., & Samsudewa, D. (2016). Evaluasi motilitas dan persentase hidup semen segar sapi PO Kebumen pejantan muda. *Jurnal Sain Peternakan Indonesia*, *11*(2), 99–107.

Butta, Crecentia Aprilinda, Gaina, Cynthia Dewi, & Foeh, Nancy D. F. K. (2021). Motilitas dan viabilitas spermatozoa babi dalam pengencer air kelapa-kuning telur ayam kampung. *Jurnal Veteriner Nusantara*, *4*(1), 3.

Derajat Keasaman Ph, Abnormalitas D. A. N., Berek, Fransiska Luruk, Dethan, Agustinus Agung, & Tahuk, Paulus Klau. (2021). Pengaruh Lama Simpan Semen Pejantan Babi Duroc Yang Diencerkan Menggunakan Pengencer Tris-Kuning Telur-Air Kelapa Muda Terhadap Nilai Viabilitas. *J. Trop. Anim. Sci. Technology*, 108.

Faizal, Muhammad. (2016). *Pengaruh penggunaan kuning telur angsa (Cignus olor) dan air kelapa muda (Cocos nucifera) terhadap kualitas sperma kambing boer dengan waktu equilibrasi yang berbeda*. Universitas Islam Negeri Maulana Malik Ibrahim.

Hoesni, Fachroerrozi. (2016). Efek penggunaan susu skim dengan pengencer tris kuning telur terhadap daya tahan hidup spermatozoa sapi. *Jurnal Ilmiah Universitas Batanghari Jambi*, *16*(3), 46–56.

Kholvi, Resha Sulthani. (2019). *Pengaruh Pemberian Glyserol Dan Ekstender Sari Buah*

Yang Berbeda Terhadap Kualitas Sperma Ikan Mas (Cyprinus Carpio) Strain Punten Pada Proses Preservasi. Universitas Brawijaya.

Kusumawati, Enike Dwi. (2022). *Sexing Spermatozoa Pada Kambing*. Media Nusa Creative (MNC Publishing).

Lestari, Try Puji, Ihsan, M. Nur, & Isnaini, Nurul. (2014). Pengaruh waktu simpan semen segar dengan pengencer andromed pada suhu ruang terhadap kualitas semen kambing Boer. *TERNAK TROPIKA Journal of Tropical Animal Production*, *15*(1), 43–50.

Mahfud, Ali, Isnaini, Nurul, Yekti, Aulia Puspita Anugra, Kuswati, Kuswati, & Susilawati, Trinil. (2019). Kualitas Spermatozoa Post Thawing Semen Beku Sperma Y Hasil Sexing Pada Sapi Limousin. *TERNAK TROPIKA Journal of Tropical Animal Production*, *20*(1), 1–7.

Muhammad, Dedi. (2018). Kualitas spermatoza sapi peranakan ongole dalam pengencer air kelapa selama penyimpanan pada suhu 4-5 oc. *Jurnal Sains Peternakan*, *6*(2), 1–9.

Nugroho, Rudy Agung, & Nur, Firman M. (2018). *Potensi bahan hayati sebagai imunostimulan hewan akuatik*. Deepublish.

Ramandhani, Sasti Nuriza, Agustini, Tri Winarni, & Suharto, Slamet. (2022). Pengaruh penambahan jenis gula yang berbeda terhadap kualitas petis dari cairan pemindangan ikan tongkol (Euthynnus affinis). *Jurnal Ilmu Dan Teknologi Perikanan*, *4*(2), 77–84.

Riyadhi, Muhammad, & Rizal, Muhammad. (2018). Viabilitas Spermatozoa Cauda Epididimis Kerbau Rawa dalam Berbagai Konsentrasi Pengencer Air Kelapa Muda dan Kuning Telur. *Acta Veterinaria Indonesiana*, *6*(1), 38–43.

Rizal, Muhammad, & Thahir, Muhammad. (2016). Daya Hidup Spermatozoa Kambing Peranakan Etawa yang Dipreservasi dengan Berbagai Jenis Pengencer. *JITRO*, *3*(3), 81–89.

Sukmawati, Eros, Arifiantini, R. I., & Purwantara, B. (2014). *Freezing capacity of sperm on various type of superior bulls.*

Yon Soepri, Ondho. (2020). *Manfaat Indogofera sp. Di Bidang Reproduksi Ternak*.

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