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PATENT SPECIFICATION

NO DRAWINGS

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COMPLETE SPECIFICATION

Preservation of Semen

We, TAKEDA YAKUHIN KOGYO KABUSHIKI KAISHA (TAKEDO CHEMICAL INDUSTRIES, LTD.) of 54, 4-chome, Jusonishino-cho, Higashiyodogawa-ku, Osaka, Japan, a body corporate organised under the laws of Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a method for increasing and/or maintaining the "viability" and "motility" of spermatozoa in semen preparations for the artificial insemination of livestock and also to a stabilized semen preparation for the artificial insemination of livestock.

In recent years, artificial insemination has been widely accepted in the reproduction of livestock such as cattle, goat and sheep. The object is not only to reproduce farm animals efficiently but also to make full use of selective semen from proven sires for the genetic improvement of breeds. For effecting artificial insemination, semen collected from the male animal is at first stored outside the body and then inseminated to the selected female animal. Therefore, reasonable operation of the artificial insemination depends fully upon the satisfactory storage of semen for a long time outside the body.

In general, the animal semen for artificial insemination is stored after it has been diluted with a suitable semen diluter, and the kind of diluter and the temperature of storage have a close connection with the "viability" and "motility" of the spermatozoa in the diluted semen, and thus with the fertilizing capacity thereof.

For the storage of the diluted semen, the metabolism of the spermatozoa must be appropriately suppressed. Lowering the storage temperature is generally adopted for this purpose. The hitherto-employed processes for storing semen for artificial insemination can be divided into two groups, i.e. the so-called liquid semen method and the so-called deep freezing method. The liquid semen method consists in diluting semen collected from male animals to about 5 to 20 times its original volume with a semen diluter and subsequently keeping the diluted semen at a temperature of 4°C, until actual use thereof.

The deep freezing method comprises diluting semen collected from male animals to 3 to 10 times its original volume with a semen diluter (the primary dilution), cooling the resultant diluted semen at 4°C, gradually for about 1 hour, further diluting the diluted semen to twice its volume with the semen diluter supplemented with glycerin as an antifreezing agent (secondary dilution), keeping the diluted semen at about 4°C. for about 6 to 18 hours in order to permeate glycerin into the spermatozoan cells (glycerin equilibrium), then freezing and storing at an ultra-low temperature of -80°C. or of -196°C, and thawing the frozen semen at a temperature of 4°C. or 37°C. before the use of the semen.

The liquid semen method, however, entails the drawbacks that the "viability" and "motility" of the spermatozoa in the diluted semen are lowered with the lapse of storage time since the metabolism of spermatozoa cannot be sufficiently inhibited, and therefore, the artificial insemination with the use of the diluted semen stored for a relatively long period beyond five days results in a low conception rate.

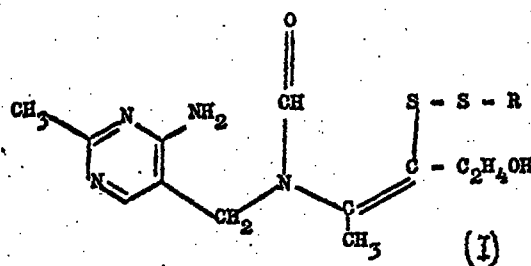
Similarly the deep freezing method entails the drawbacks that the "viability" and "motility" of spermatozoa in the thawed semen obtained by thawing the frozen semen lowers with the lapse of time although the "viability" and "motility" of the spermatozoa in the frozen semen are kept at rather stable conditions throughout the period of freezing storage.

Thus, it has been one of the most important desiderata in the field of stock-breeding to establish a method for keeping spermatozoa in the diluted semen and the thawed semen from the frozen semen viable for a long time.

It is an object of the present invention to provide a method for increasing and/or maintaining the "viability" and "motility" of spermatozoa in a semen preparation for artificial insemination of livestock. Another object of the present invention is to provide a stabilized semen preparation suitable for a long storage of the semen. Other objects will be apparent from the detailed description of the present invention hereinafter provided.

In accordance with one embodiment of the present invention, we have found that the "viability" and "motility" of the spermatozoa of livestock, especially of ruminant livestock such as cattle, goat, sheep, in preparations, e.g. the diluted semen and the thawed semen from the frozen semen, can be increased and/or maintained so that the fertilizing capacity of the spermatozoa can be increased and/or maintained for an extended period of time by the presence of a small amount of certain thiamine derivatives.

The thiamine derivative to be contained in the semen preparation is an unsymmetrical thiamine organic disulfide derivative of the following formula:



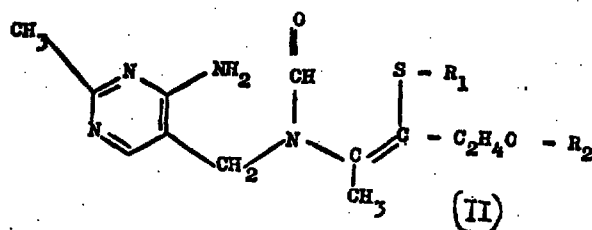
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in which R is a substituted or unsubstituted alkyl group, e.g.:

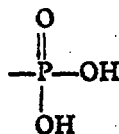
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Compound	R
thiamine tetrahydrofurfuryl disulphide	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_2 - \text{CH}_2 - \text{O} - \text{CH}_2 \\ \\ \text{CH}_2 \end{array}$
thiamine allyl disulfide	$-\text{CH}_2\text{CH}=\text{CH}$
thiamine 2-hydroxyethyl disulfide	$-\text{C}_2\text{H}_4\text{OH}$
thiamine (7-methoxycarbonyl-3-acetylthioheptyl) disulfide	$\begin{array}{c} \text{CH}_3 \\ \\ -\text{CH}_2 - \text{CH}(\text{CH}_2)_4\text{COOCH}_3 \\ \\ \text{SCOCH}_3 \end{array}$
thiamine propyl disulfide	$-\text{C}_3\text{H}_7$

or a thiamine derivative of the following formula:



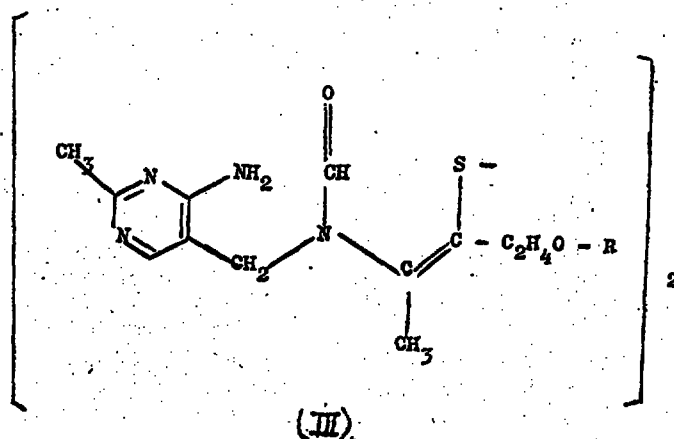
in which R_1 and R_2 , which may be the same or different, represent carboxylic acid acyl, carboalkoxy or, taken together, the cyclocarbo group $>C=O$ or R_2 may be a phosphono group, where phosphono means



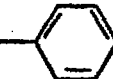
e.g.

Compounds	R_1	R_2
O,S-dibenzoyl-thiamine	$-CO-$	$-CO-$
O,S-diacetyl thiamine	$-CO-CH_3$	$-CO-CH_3$
S-acetyl thiamine O-monophosphate	$-CO-CH_3$	$\begin{array}{c} O \\ \\ -P-OH \\ \\ OH \end{array}$
O,S-dicarboethoxy-thiamine	$-COOC_2H_5$	$-COOC_2H_5$
O,S-cyclocarbo thiamine	$\begin{array}{c} -R_1 \\ \diagdown \quad \diagup \\ R_2 = \end{array}$	$\begin{array}{c} \diagdown \quad \diagup \\ C=O \end{array}$

or a bithiamine-disulfide derivative of the following formula:



in which R is H or carboxylic acid acyl, e.g.:

Compounds	R
bisthiamine-disulfide	H
bis(O-benzoylthiamine)-disulfide	- CO - 

The said thiamine derivatives may be employed as free compounds or corresponding salts with a suitable acid such as hydrochloric acid and nitric acid. In the method of the present invention one compound or a mixture of more than one of these thiamine derivatives is added to the semen preparation for artificial insemination. From the viewpoint of the effect of increasing and/or maintaining the "viability" and "motility" of the spermatozoa in the semen preparation, the thiamine derivative is used in such an amount as from 0.001 to 0.1 g per 100 c.c., preferably 0.006 to 0.01 g per 100 c.c. of the semen preparation.

The semen preparation to be incorporated with the thiamine derivative may be any of the conventional semen preparations for artificial insemination of livestock. That is to say, according to the method of the present invention, either the diluted semen in the above-mentioned liquid semen method or the thawed semen obtained by thawing the frozen semen in the above-mentioned deep freezing method can have the "viability" and "motility" of its spermatozoa increased and/or maintained. This effect of increasing and/or maintaining the viability and "motility" of the spermatozoa can be attained by adding a thiamine derivative to any conventional semen preparation before use. In the case of the diluted semen in the liquid semen method, the thiamine derivative may be added to the semen diluter with which the semen is diluted, to the diluted semen just after the dilution, or to the diluted semen under the storage at about 4°C. In a case of the thawed semen in the deep freezing method, the thiamine derivative may be added to the semen in any of the steps of the second dilution, of the glycerin equilibrium end of the storage at about 4°C after thawing. Most practically, the object of the present invention can be attained by diluting the semen with a semen diluter containing the thiamine derivative, which is prepared by adding the derivative to any of hitherto known diluters. The concentration of the thiamine in the semen diluter is 0.001 to 0.1 g per 100 c.c.s., preferably 0.006 to 0.01 g per 100 c.c.s. of the diluter.

The semen diluter to be employed in the present invention may be any of the ordinary diluters known in the current art. In general, the diluters may comprise, as a basal component, egg yolk, egg yolk product such as lecithin, milk or milk product such as defatted milk or casein, and additionally may contain inorganic and organic buffering salts, and energy sources such as fructose and amino acid, all of which are familiar in the veterinary and animal husbandry art, and are commonly chosen depending upon the factors such as kind of the livestock. As the examples of such semen diluter there may be enumerated an egg yolk diluter such as egg yolk-citrate buffer solution, egg yolk-phosphate buffer solution, a milk diluter which is prepared by heating whole milk or skim milk.

Thus prepared semen preparation containing the thiamine derivative is very high in the "viability" and "motility" of the spermatozoa therein and therefore the fertilizing capacity of the spermatozoa in the semen preparation can persist after many days of storage.

The following examples are merely intended to illustrate presently preferred embodiments of the methods and compositions of the present invention and not to restrict the scope of the latter.

Throughout the present specification as well as in the following examples, the abbreviations "ml" and "°C" respectively refer to "milliliters" and "degrees centigrade", and percentages are g per 100 c.c.s. unless otherwise described:

In the following examples the "viability" and the "motility" of spermatozoa are shown by the following references:

- +.+.+: Spermatozoa with very actively progressive motion.
 ++: Spermatozoa with actively progressive motion.
 +: Spermatozoa with progressive motion.
 ±: Rotative or pendulum-like motile spermatozoa.
 +++~++: Spermatozoa with a motion between said +++ and ++.
 ++~+: Spermatozoa with a motion between said ++ and +.
 +~±: Spermatozoa with a motion between said + and ±.

Percentages which are described in front of said references mean percentage of spermatozoa with such motion to all spermatozoa in the semen preparation. For example, 85+++ means that percentage of spermatozoa with very actively progressive motion and percentage of spermatozoa with actively progressive motion to all spermatozoa in the semen preparation are 85 and 5 respectively.

The "viability" indices of spermatozoa in the semen preparation are shown by the values calculated from the following formula:

$$\text{"Viability" index} = \frac{100a + 75b + 50c + 25d}{100}$$

wherein a: Percentage of +++ to all spermatozoa in the semen preparation.
 b: Percentage of ++.
 c: Percentage of +.
 d: Percentage of ±

EXAMPLE 1

Bovine semen collected from 2 bulls is diluted 10-fold at the body temperature with an egg yolk-citrate buffer solution containing 2.4% of sodium citrate and 20% of fresh yolk and 0.015625%, 0.0078125% or 0.0020000% of thiamine propyl disulfide. Each of the diluted semen samples is cooled to 4°C. and stored at even temperature for 7 days. The "viability" and "motility" of the spermatozoa in one drop of each of the diluted semen samples is examined microscopically at 4°C. The results are shown in the following Table I.

TABLE 1

Bulls	Concentration of thiamine propyl disulfide in the semen diluter (%)	Storage period (days)							
		Immediately after the dilution	1	2	3	4	5	6	7
A	0.0156250	85+++ 5+	80+++ 5+	75+++ 5+	70+++ 5+	65+++ 5+	65+++ 5+	50+++~+++ 5+	30+++~+ 5+
	0.0078125	80+++ 5+	80+++ 5+	75+++ 5+	65+++ 5+	65+++ 5+	60+++~+++ 5+	50+++~+++ 5+	40+++ 10+
	0.0020000	75+++ 5+	70+++ 5+	65+++ 5+	55+++ 5+	50+++~+++ 5+	40+++~+++ 5+	40+++~+++ 5+	30+ 5±
	0 (Control)	70+++ 5+	70+++ 10+	70+++ 5+	55+++ 5+	50+++ 5+	40+++~+++ 5+	40+++ 10+	30+++~+ 5+
B	0.0156250	85+++ 5+	80+++ 5+	75+++ 5+	75+++ 5+	50+++~+++ 5+	40+++~+++ 5+	20+~±	et*
	0.0078125	85+++ 5+	80+++ 5+	75+++ 5+	70+++ 5+	65+++ 5+	60+++ 10+	50+++~+++ 5+	40+++ 5+
	0.0020000	70+++ 5+	70+++ 5+	70+++ 5+	55+++ 5+	40+++ 5+	30+ 10±	20+ 10±	20±
	0 (Control)	70+++ 5-	70+++ 10	70+++ 5+	50+++ 5+	40+++~+++ 5+	30+++~+ 10±	30+++~±	20±

et*: "motile" but unnumbered.

EXAMPLE 2

Rovine semen collected from one bull is diluted 10-fold at the body temperature of the bull, 39°C, and then diluted 10-fold at 20°C.

EXAMPLE 2

Bovine semen collected from one bull is diluted 10-fold at the body temperature with an egg yolk-citrate buffer solution containing 2.4% of sodium citrate and 20% of fresh yolk. The diluted semen is stored at 4°C. After two days storage, thiamine tetrahydrofurfuryl disulfide, thiamine tetrahydrofurfuryl disulfide hydrochloride, thiamine propyl disulfide and thiamine propyl disulfide hydrochloride is added to the diluted semen in a final concentration of 1%, 0.5% or 0.0625%.

The diluted semen is further stored at 4°C for 5 days. Throughout the period of the storage, the "viability" and "motility" of spermatozoa in the diluted semen is examined microscopically. The results are shown in the following Table 2.

The use of amounts of derivatives outside the scope of this invention in this example is so that the results obtained may be used for comparative purposes.

TABLE 2

Thia- mine deriva- tives	Concen- tration (%)	Storage period (days)						
		1	2		3	4	5	6
		Imme- diately after the dilution	Before the addi- tion	Imme- diately after the addition				
TTFD*	1.0000	85+++	70+++ 5+	85+++	0			
	0.5000	"	"	85+++	20+~±	0		
	0.0625	"	"	75+++ 5+	80+++	70+++ 5+	55+++~++ 5+	40+++~+ 5+
TTFD* hydro- chloride	1.0000	"	"	85+++	et*	0		
	0.5000	"	"	80+++	30+~±	0		
	0.0625	"	"	75+++ 5+	75+++ 5+	60+++ 10+	40+++~+ 10+	35+++~+ 5+

TABLE 2 (continued)

Storage period (days)										
Thia- mine deriva- tives	Concen- tration (%)	Imme- diately after the dilution	1	2		3	4	5	6	7
				Before the addi- tion	Imme- diately after the addition					
TPD**	1.0000	"	"	"	90+++	0				
	0.5000	"	"	"	85+++	10±	0			
	0.0625	"	"	"	80+++	90++	75+++ 5+	70+++ 10+	60+++~+++ 5+	50+++~+++ 5+
TPD** hydro- chloride	1.0000	"	"	"	85+++	0				
	0.5000	"	"	"	80+++ 5+	10+~±	0			
	0.0625	"	"	"	75+++ 5+	90+++	75+++ 5+	70+++ 5+	65+++ 5+	60+++ 5+
Control A	0	"	"	"	70+++ 5+	70+++	60+++~+++ 5+	45+++~+ 5+	40+++ 5+	40+ 5±
	0	"	"	"	70+++ 5+	70+++	55+++~+++ 10+	45+++~+ 5+	40+ 5±	20+~±

TTFD*: thiamine tetrahydrofurfuryl disulfide

TPD**: thiamine propyl disulfide

EXAMPLE 3

Bovine semen collected from one bull is diluted 10-fold at the body temperature with an egg yolk-citrate buffer solution containing 2.4% of sodium citrate and 20% of fresh yolk. The diluted semen is stored at 4°C. After four days storage, thiamine tetrahydrofurfuryl disulfide, thiamine tetrahydrofurfuryl disulfide hydrochloride, thiamine propyl disulfide and thiamine propyl disulfide hydrochloride are added to the diluted semen in concentrations described in the following Table 3. The diluted semen is further stored at 4°C for 3 days. Throughout the period of the storage, the viability and "motility" of spermatozoa in the diluted semen are examined microscopically. The results are shown in the following Table 3.

The use of amounts of derivatives outside the scope of this invention in the example is so that the results may be used for comparative purposes.

TABLE 3

Storage period (days)										
Concentration of test compounds in the diluted semen (%)	Imme- diately after the dilu- tion	1	2	3	4			5	6	7
					Before the addition of test com- pounds	Imme- diately after the addition of test com- pounds				
Deriva- tives	0.062500	85+++	75+++	65+++	60+++ 5+	65+++ 5+	75+++ 5+	75+++ 5+	75+++	60+++~+++ 5+
	0.031250	"	"	"	"	60+++ 10+	70+++ 5+	65+++~+++ 5+	65+++~+++ 5+	
	0.015625	"	"	"	"	60+++ 10+	60+++~+++ 10+	55+++~+++ 5+	55+++ 5	
TTFD*	0.125000	"	"	"	"	70+++ 5+	80+++ 5+	40+ 5±	40+ 5±	0
	0.062500	"	"	"	"	65+++ 5+	75+++ 5+	75+++	75+++	70+++~+++ 5+
	0.031250	"	"	"	"	65+++ 5+	65+++ 5+	60+++ 5+	60+++	60+++~+++ 5+
hydro- chlor- ide-	0.015625	"	"	"	"	60+++ 5+	60+++ 10+	60+++	60+++	60+++~+++ 5+

TABLE 3 (continued)

Storage period (days)							

TABLE 3 (continued)
Storage period (days)

Derivatives	Concentration of test compounds in the diluted semen (%)	Immediately after the dilution	1	2	3	4		5	6	7
						Before the addition of test compounds	Immediately after the addition of test compounds			
TPD**	0.062500	"	"	"	"	"	65+++ 5+	80+++ 5+	75+++	65+++~+++ 5+
	0.031250	"	"	"	"	"	65+++ 5+	70+++ 5+	70+++	70+++~+++ 5+
	0.015625	"	"	"	"	"	60+++ 5+	65+++~+++ 5+	60+++~+++ 5+	50+++ 5+
TPD** hydrochloride	0.062500	"	"	"	"	"	65+++ 5+	80+++ 5+	80+++	75+++
	0.031250	"	"	"	"	"	60+++ 10+	70+++ 5+	65+++ 10+	60+++~+++ 5+
	0.015625	"	"	"	"	"	60+++ 5+	60+++~+++ 5+	55+++~+++ 5+	50+++ 10+
Control A	0						60+++ 5+	50+++~+++ 10+	50+++ 5+	50+++~+
Control B	0	"	"	"	"	"	60+++ 5+	50+++~+++ 10+	50+++ 5+	50+++~+

TPD*: thiamine tetrahydrofurfuryl disulfide TPD**: thiamine propyl disulfide

EXAMPLE 4

Goat semen collected from one sire is diluted 5-fold at the body temperature with a physiological saline solution and the mixture is subjected to centrifugation to get rid of seminal plasma. After decanting the supernatant, sedimenting spermatozoa is resuspended into an egg yolk-citrate buffer solution containing 2.4% of sodium citrate and 20% of fresh yolk in the same volume as the original semen.

The resulting semen is diluted 10-fold at the body temperature with an egg yolk-citrate buffer solution of the same composition as described above. The diluted is stored at 4°C. After two days storage, thiamine tetrahydrofurfuryl disulfide, thiamine propyl disulfide and thiamine propyl disulfide hydrochloride are added to the diluted semen in concentrations described in the following Table 4. The diluted semen is further stored at 4°C. for 5 days. Throughout the period of the storage, the "viability" and "motility" of the spermatozoa in the diluted semen are examined microscopically. The results are shown in the following Table 4.

The use in this example of amounts of derivative outside the scope of this invention is so that the results may be used for comparative purposes.

TABLE 4

Thia- mine deriva- tives	Concentration of test compounds in the diluted semen (%)	Storage period (days)						
		2		3	4	5	6	7
		Before the addition of test com- pounds	Imme- diately after the addition of test com- pounds					
TTFD*		1						
	1.0000	55+++ 5+	50+++ 5+	75+++ 5+	0	0	0	0
	0.5000	"	"	75+++ 5+	0	0	0	0
TPD**	0.0625	"	"	60+++ 5+	65+++ 5+	55+++~+++ 5+	45+++ 5+	25+++ 10+
	1.0000	"	"	75+++ 5+	0	0	0	0
	0.5000	"	"	75+++ 5+	0	0	0	0
	0.0625	"	"	60+++ 5+	70+++ 5+	65+++ 5+	60+++~+++ 5+	55+++~+++ 5+

TABLE 4 (continued)

Thia- mine deriva- tives	Concentration of test compounds in the diluted semen (%)	Storage period (days)						
		2		3	4	5	6	7
		Before the addition of test com- pounds	Imme- diately after the addition of test com- pounds					
TPD**	1.0000	"	75+++ 5+	0	0	0	0	0
hydro- chloride	0.5000	"	75+++ 5+	0	0	0	0	0
	0.0625	"	60+++ 5+	75+++ 5+	70+++ 5+	65+++ 5+	60+++~+++ 5+	50+++~+++ 5+
Control A	0	"	50+++ 5+	45+++~+++ 5+	40+++ 5+	35+++ 5+	30+++ 5+	20+++~+ 5+
Control B	0	"	50+++ 5+	45+++~+++ 5+	40+++ 5+	35+++ 5+	30+++ 5+	20+++~+ 5+

TTFD*: thiamine tetrahydrofurfuryl disulfide

TPD**: thiamine propyl disulfide

EXAMPLE 5

Bovine semen collected from 3 bulls is diluted 10-fold at the body temperature with a milk diluter which is prepared by heating fresh milk at 92°C for 10 minutes to inactivate harmful materials to spermatozoan "viability" and subsequently removing therefrom milk fat by centrifugation for 30 minutes at 3000 r.p.m. To the diluted semen is added thiamine propyl disulfide hydrochloride in concentrations of 0.00313% and 0.00156%. Each of the diluted semen is stored at 4°C for 7 days. The "viability" and "motility" of spermatozoa in the diluted semen is examined microscopically. The results are shown in the following Table 5.

TABLE 5

Bulls	Concentration of thiamine propyl disulfide hydrochloride in the diluted semen (%)	Storage time (days)								
		0	1	2	3	4	5	6	7	
A	0(Control)	60+++	50+++~+++	40+++~+++	40+++~+++	35+++	20+++~+	15+~±	0	
		5+	10+	5+	5+	5+	5+			
	0.00313	65+++	60+++	50+++~+++	40+++~+++	40+++	10+	5±	0	
		5+	5+	5+	5+	5+	10±			
	0.00156	65+++	55+++	50+++~+++	45+++~+++	35+++	15+~±	5±	0	
		5+	10+	5+	5+	5+				

TABLE 5 (continued)

Bulls	Concentration of thiamine propyl disulfide hydrochloride in the diluted semen (%)	Storage time (days)							
		0	1	2	3	4	5	6	7
B	0(Control)	65+++ 5+	60+++ 5+	40+++~+++ 10+	40+++~+++ 10+	30+++ 5+	25+++ 10+	20+++~+ 10+	20+~±
		70+++ 5+	65+++ 5+	55+++ 10+	50+++ 10+	40+++~+++ 5+	25+++ 10+	15+++~+ 10+	et*±
	0.00313	70+++ 5+	60+++ 10+	55+++ 5+	50+++~+++ 10+	30+++ 5+	25+++ 5+	20+~ 5+	et*±
		70+++ 5+	60+++ 5+	50+++ 5+	40+++ 5+	20+++~+ 10+	10+ 10±	et*±	0
	0.00156	70+++ 5+	65+++ 5+	60+++ 5+	50+++ 5+	30+++ 10+	15+++~+ 10+	10+~±	et*±
		70+++ 5+	65+++ 5+	60+++ 5+	45+++~+++ 5+	30+++ 10+	20±--+ 5±	15+~±	et*±
C	0(Control)	70+++ 5+	60+++ 5+	50+++ 5+	40+++ 5+	20+++~+ 10+	10+ 10±	et*±	0
		70+++ 5+	65+++ 5+	60+++ 5+	50+++ 5+	30+++ 10+	15+++~+ 10+	10+~±	et*±
	0.00313	70+++ 5+	65+++ 5+	60+++ 5+	50+++ 5+	30+++ 10+	15+++~+ 10+	10+~±	et*±
		70+++ 5+	65+++ 5+	60+++ 5+	45+++~+++ 5+	30+++ 10+	20±--+ 5±	15+~±	et*±
	0.00156	70+++ 5+	65+++ 5+	60+++ 5+	50+++ 5+	30+++ 10+	15+++~+ 10+	10+~±	et*±
		70+++ 5+	65+++ 5+	60+++ 5+	45+++~+++ 5+	30+++ 10+	20±--+ 5±	15+~±	et*±

et*: "motile" but unnumbered.

EXAMPLE 6

Bovine semen collected from 3 bulls is diluted 10-fold at the body temperature with a milk diluter prepared after the manner described in Example 5. The diluted semen is stored at 4°C. After two days storage, thiamine propyl disulfide hydrochloride is added to the diluted solution in concentrations of 0.00313% and 0.00156%. Each of diluted semen samples is further stored at 4°C for 5 days. The "viability" and "motility" of spermatozoa in the respective diluted semen samples is shown in the following Table 6.

TABLE 6

Bulls	Concentration of thiamine propyl disulfide hydrochloride in the diluted semen (%)	Storage period (days)						
		2		3	4	5	6	7
		Before the addition	Immediately after the addition					
A	0 (Control)	0	1	70+++ 5+	60+++ 5+	55+++ 5+	50+++~+++ 5+	40+++~+++ 10+
		70+++ 5+	60+++ 5+					
	0.00313	"	"	70+++ 5+	60+++ 5+	45+++~+++ 10+	20+++~+ 10+	20+++~+ 5±
		"	"					
	0.00156	"	"	70+++ 5+	50+++ 10+	40+++~+++ 10+	20+++ 10+	10+ 10±
		"	"					

TABLE 6 (continued)

Storage period (days)										
Concentration of thiamine propyl disulfide hydrochloride in the diluted semen (%)	0	1	2		3	4	5	6	7	
			Before the addition	Immediately after the addition						
Bulls										
	0(Control)	60+++ 5+	55+++ 5+	50+++ 10+	45+++~+++ 5+	30+++ 5+	20+++~+ 10+	20+++~+ 5±	20+++~+ 5±	
	0.00313	"	"	55+++ 5+	55+++ 5+	50+++~+++ 10+	35+++ 10+	20+++~+ 10+	20+++~+ 5±	
	0.00156	"	"	50+++ 10+	55+++ 5+	40+++~+++ 10+	40+++ 5+	30+++~+ 5+	20+++~+ 10+	
C	0(Control)	70+++ 5+	60+++ 5+	45+++~+++ 10+	40+++~+++ 10+	40+++~+++ 5+	30+++ 10+	25+++~+ 10+	15+++~+ 10±	
	0.00313	"	"	45+++~+++ 10+	60+++ 10+	55+++ 5+	25+++~+ 5+	15+++~+ 10+	10+++~+ 10±	
	0.00156	"	"	45+++~+++ 10+	60+++ 5+	40+++~+++ 10+	20+++~+ 10+	20+++~+ 10±	10+++~+ 10±	

EXAMPLE 7

Bovine semen collected from 2 bulls is diluted 10-fold at the body temperature with a milk diluter prepared after the manner described in Example 5. The diluted semen is stored at 4°C. After four days storage, thiamine propyl disulfide hydrochloride is added to the diluted solution in concentrations of 0.00313% and 0.00156%. Each of the diluted semen samples is further stored at 4°C for 3 days. The "viability" and "motility" of spermatozoa in the respective diluted semen samples is shown in the following Table 7.

TABLE 7

Storage period (days)										
Bulls	Concentration of thiamine propyl disulfide hydrochloride added in the diluted semen (%)	4								
		0	1	2	3	Before the addition	Immediately after the addition	5	6	7
A	0(Control)	70+++ 5+	60+++ 5+	45+++ ~+++ 10+	40+++ ~+++ 10+	40+++ ~+++ 5+	40+++ ~+++ 5+	40+++ ~+++ 5+	20+++ ~+++ 10±	10+ 10±
	0.00313	"	"	"	"	"	40+++ ~+++ 5+	40+++ ~+++ 10+	25+++ 10+	10+++ ~+++ 10±
	0.00156	"	"	"	"	"	40+++ ~+++ 5+	40+++ ~+++ 5+	30+++ 5+	20+++ ~+++ 5±
B	0(Control)	70+++ 5+	60+++ 5+	50+++ 10+	50+++ 5+	50+++ 5+	50+++ 5+	45+++ ~+++ 5+	40+++ ~+++ 5+	30+++ 5+
	0.00313	"	"	"	"	"	50+++ 5+	50+++ 10+	40+++ ~+++ 5+	30+++ 5+
	0.00156	"	"	"	"	"	50+++ 5+	50+++ 10+	40+++ ~+++ 5+	30+++ 5+

EXAMPLE 8

Bovine semen collected from 2 bulls is diluted 5-fold at the body temperature with an egg yolk citrate-buffer solution containing 2.4% of sodium citrate and 20% of fresh yolk, and the resulting diluted semen is cooled to 4°C gradually for 1 hour. The diluted semen is further diluted 2-fold at 4°C with an egg yolk-citrate buffer solution containing 2.4% of sodium citrate, 20% of fresh yolk and 14.0% of glycerin. To the resultant diluted semen are added thiamine propyl disulfide and thiamine propyl disulfide hydrochloride in concentrations described in the following Table 8. And then the diluted semen is kept standing at 4°C for 10 hours. 1ml. each of the diluted semen samples is put into ampoules of 2ml. capacity and the ampoules are closed. After being frozen and stored in a liquid-nitrogen refrigerator at -196°C for 3 days, each of the ampouled samples is thawed at 4°C and stored at even temperature for 24 hours. The change of the "viability" index of the respective thawed semen samples is shown in the following Table 8.

TABLE 8

Bulls	Thia- mine deriva- tives	Concentra- tion of test com- pound in the thawed semen	Viability index						
			Before the refrigeration		After thawing				
			Before the addi- tion of test com- pounds	Just after the addi- tion of test com- pounds	Just after thawing	4 hrs. after thaw- ing	12 hrs. after thaw- ing	24 hrs. after thaw- ing	
A	Thiamine propyl disul- fide	0.04545	75.0	80.0	62.5	57.5	52.9	33.3	
		0.02270	"	75.0	62.5	62.5	52.9	48.3	
		0.01136	"	75.0	67.5	67.5	62.5	48.3	
		0.00568	"	75.0	67.5	67.5	62.5	52.9	
		0.00284	"	75.0	57.5	57.5	52.9	48.3	
		0.00142	"	75.0	57.5	57.5	48.3	48.3	
	Thiamine propyl disul- fide hydro- chloride	0.04545	"	80.0	62.5	52.9	52.9	35.8	
		0.02270	"	75.0	62.5	52.9	52.9	44.2	
		0.01136	"	75.0	62.5	62.5	62.5	48.3	
		0.00568	"	75.0	62.5	62.5	57.5	43.8	
	Control	0.00284	"	75.0	62.5	57.5	52.9	48.3	
		0.00142	"	75.0	57.5	57.5	52.9	48.3	
		0	"	—	57.5	52.9	40.0	33.3	

TABLE 8 (continued)

				Viability index				
				Before the refrigeration		After thawing		
Bulls	Thia- mine deriva- tives	Concentra- tion of test com- pound in the thawed semen	Before the addi- tion of test com- pounds	Just after the addi- tion of test com- pounds	Just after thawing	4 hrs. after thaw- ing	12 hrs. after thaw- ing	24 hrs. after thaw- ing
B	Thiamine propyl disul- fide	0.04545	80.0	80.0	62.5	62.5	52.9	48.3
		0.02270	"	80.0	62.5	67.5	62.5	52.9
		0.001136	"	80.0	62.5	67.5	67.5	62.5
		0.00568	"	80.0	57.5	67.5	67.5	62.5
		0.00284	"	80.0	57.5	52.9	62.5	52.9
		0.00142	"	80.0	57.5	52.9	52.9	52.9
	Thiamine propyl disul- fide hydro- chloride	0.04545	"	80.0	62.5	67.5	48.3	48.3
		0.02270	"	80.0	62.5	87.5	62.5	48.3
		0.01136	"	80.0	62.5	67.5	67.5	57.5
		0.00568	"	80.0	57.5	62.5	62.5	57.5
	Control	0.00284	"	80.0	57.5	52.9	57.9	52.5
		0.00142	"	80.0	57.5	52.9	52.9	50.4
		0	"	—	57.5	52.9	52.9	30.4

EXAMPLE 9

Goat semen collected from 2 sires is diluted 5-fold at the body temperature with a physiological saline solution and the resultant mixture is subjected to centrifugation to get rid of seminal plasma. After decanting the supernatant, sedimenting spermatozoa is resuspended into an egg yolk-citrate buffer solution containing 2.4% of sodium citrate and 20% of fresh yolk in the same volume as the original semen. The resultant semen is diluted 5-fold at the body temperature with an egg yolk-citrate buffer solution of the same composition as described above. After being cooled to 4°C gradually for 1 hour, the diluted semen is further diluted 2-fold with an egg yolk-citrate buffer solution containing 2.4% of sodium citrate and 20% of fresh yolk, 14% of glycerin. To the resultant diluted semen are added thiamine propyl disulfide and thiamine propyl disulfide hydrochloride in concentrations described in the following Table 9. And then the diluted semen is kept standing at 4°C for 10 hours. 1ml. each of the diluted semen samples is put into ampoules of 2ml. capacity and the ampoules are closed. After being frozen and stored in a dry-ice refrigerator at -79°C for 3 days, each of the ampouled samples are thawed at 4°C and stored at even temperature for 24 hours. The change of "viability" index of the respective thawed semen samples is shown in the following Table 9.

Control	0	"	—	57.5	52.9	52.9	30.4
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TABLE 9

Bulls	Thiamine derivatives	Concentration of test compounds in the thawed semen	Viability index					
			Before the refrigeration		After thawing			
			Before the addition of test compounds	Just after the addition of test compounds	Just after thawing	4 hrs. after thawing	12 hrs. after thawing	24 hrs. after thawing
A	Thiamine propyl disulfide	0.02270	80.0	85.0	57.5	57.5	62.5	30.0
		0.01136	"	80.0	52.5	57.5	67.5	48.3
		0.00568	"	80.0	39.2	52.4	62.5	57.5
		0.00284	"	80.0	39.2	47.5	48.3	48.3
		0.00142	"	80.0	34.6	39.2	33.3	30.0
	Thiamine propyl disulfide hydrochloride	0.02270	"	85.0	57.5	57.5	57.5	20.4
		0.001136	"	80.0	43.7	57.5	57.5	48.3
		0.00568	"	80.0	43.7	52.5	57.5	57.5
		0.00284	"	80.0	43.7	43.7	31.7	28.8
		0.00142	"	80.0	43.7	43.7	31.7	36.3
	Control	0	"	80.0	31.7	31.7	31.7	17.5

TABLE 9 (continued)

Bulls	Thiamine derivatives	Concentration of test compounds in the thawed semen	Viability index					
			Before the refrigeration		After thawing			
			Before the addition of test compounds	Just after the addition of test compounds	Just after thawing	4 hrs. after thawing	12 hrs. after thawing	24 hrs. after thawing
B	Thiamine propyl disulfide	0.02270	"	80.0	62.5	62.5	62.5	30.0
		0.01136	"	80.0	57.5	57.5	62.5	57.5
		0.00568	"	80.0	52.9	52.9	52.9	44.2
		0.00284	"	80.0	52.9	52.9	52.9	44.2
		0.00142	"	80.0	52.9	52.9	48.3	30.0
	Thiamine propyl disulfide hydrochloride	0.02270	"	80.0	57.5	62.5	62.5	44.2
		0.01136	"	80.0	57.5	62.5	62.5	57.5
		0.00568	"	80.0	57.5	62.5	62.5	57.5
		0.00284	"	80.0	57.5	52.9	52.9	44.2
		0.00142	"	80.0	57.5	52.9	48.3	30.0
	Control	0	"	80.0	52.9	52.9	40.0	26.7

...	31.7	31.7	31.7	31.7	17.5
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EXAMPLE 10

5 Bovine semen collected from 2 bulls is diluted 5-fold at the body temperature
with an egg yolk-citrate buffer solution containing 2.4% of sodium citrate and 20%
of fresh yolk, and the resulting diluted semen is cooled to 4°C gradually for 1 hour.
10 The diluted semen is further diluted 2-fold at 4°C with an egg yolk-citrate buffer
solution containing 2.4% of sodium citrate, 20% of fresh yolk and 14% of glycerin.
After being kept standing at 4°C for 10 hours, the diluted semen samples is put into
ampoules and the ampoules are closed. After being frozen and stored in a liquid-
nitrogen refrigerator at -196°C for 3 days, each of the ampouled samples is thawed
15 at 4°C. To the thawed semen samples are added thiamine propyl disulfide and
thiamine propyl disulfide hydrochloride in concentrations described in the following
Table 10.
And then the thawed semen samples are further stored at 4°C for 24 hours.
The change of the livability index of the respective thawed semen samples is shown
in the Table 10.

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TABLE 10

Bulls	Thia- mine deriva- tives	Concentra- tion of test com- pounds in the thawed semen	Viability index					
			Before the re- frigera- tion	After thawing				
				Just after thaw- ing	Just after the addi- tion	4 hrs. after the addi- tion	12 hrs. after the addi- tion	24 hrs. after the addi- tion
propyl	Thiamine propyl disul- fide	0.04545	80.0	52.9	62.5	62.5	52.9	40.4
		0.02270	"	"	62.5	62.5	52.9	52.9
		0.01136	"	"	62.5	67.5	52.9	52.9
		0.00568	"	"	62.5	62.5	62.5	57.5
		0.00284	"	"	57.5	57.5	52.9	48.3
		0.00142	"	"	57.5	57.5	40.0	40.0
A	Thiamine propyl disul- fide hydro- chloride	0.04545	"	"	62.5	67.5	57.5	40.4
		0.02270	"	"	62.5	67.5	57.5	48.3
		0.01136	"	"	62.5	72.5	62.5	48.3
		0.00568	"	"	57.5	62.5	62.5	57.5
		0.00284	"	"	57.5	57.5	57.5	52.9
		0.00142	"	"	57.5	57.5	48.3	43.7
	Control	0	"	"	52.9	52.9	35.8	35.8

TABLE 10 (continued)

Bulls	Thia- mine deriva- tives	Concentra- tion of test com- pounds in the thawed semen	Viability index					
			Before the re- frigera- tion	After thawing				
				Just after thaw- ing	Just after the addi- tion	4 hrs. after the addi- tion	12 hrs. after the addi- tion	24 hrs. after the addi- tion
B	Thiamine propyl disul- fide	0.04545	"	62.5	67.5	72.5	52.9	48.3
		0.02270	"	"	67.5	72.5	52.9	52.9
		0.01136	"	"	67.5	72.5	62.5	62.5
		0.00568	"	"	57.5	67.5	72.5	62.5
		0.00284	"	"	57.5	62.5	62.5	62.5
		0.00142	"	"	57.5	62.5	57.5	48.3
	Thiamine propyl disul- fide hydro- chloride	0.04545	"	"	72.5	72.5	62.5	48.3
		0.02270	"	"	72.5	72.5	67.5	60.0
		0.01136	"	"	72.5	72.5	67.5	62.5
		0.00568	"	"	67.5	67.5	67.5	62.5
	Control	0.00284	"	"	67.5	67.5	62.5	57.5
		0.00142	"	"	62.5	62.5	57.5	48.3
		0	"	"	62.5	62.5	44.2	40.2

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EXAMPLE 11

Goat semen collected from 2 sires is diluted 5-fold at the body temperature with a physiological saline solution and the resultant mixture is subjected to centrifugation to get rid of seminal plasma. After decanting the supernatant, sedimenting spermatozoa is resuspended into an egg yolk-citrate buffer solution containing 2.4% of sodium citrate and 20% of fresh yolk in the same volume as the original semen. The resultant semen is diluted 5-fold at the body temperature with an egg yolk-citrate buffer solution of the same composition as described above. After being cooled to 4°C gradually for 1 hour. The diluted semen is further diluted 2-fold with an egg yolk-citrate buffer solution containing 2.4% of sodium citrate, 20% of fresh yolk and 14% of glycerin. After being kept standing at 4°C for 11 hours, 1ml. each of the diluted semen samples is put into ampoules and the ampoules are closed. After being frozen and stored in a liquid-nitrogen refrigerator at -196°C for 3 days, each of the ampouled samples is thawed at 4°C. To the thawed semen samples are added thiamine propyl disulfide or thiamine propyl disulfide hydrochloride in concentrations described in the following Table 11, and then the thawed semen samples are stored at 4°C for 24 hours. The change of the viability index of the respective thawed samples is shown in the Table 11.

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TABLE 11

Bulls	Thia- mine deriva- tives	Concentra- tion of test com- pounds in the thawed semen	Viability index					
			Before the refri- gera- tion	After thawing				
				Just after thaw- ing	Just after the addi- tion	4 hrs. after the addi- tion	12 hrs. after the addi- tion	24 hrs. after the addi- tion
A	Thiamine propyl disul- fide	0.02270	80.0	27.5	57.5	57.5	57.5	22.5
		0.01136	"	"	47.5	52.5	57.5	48.3
		0.00568	"	"	39.5	39.5	48.3	48.3
		0.00284	"	"	36.7	36.7	43.7	26.3
		0.00142	"	"	36.7	36.7	43.7	26.3
	Thiamine propyl disul- fide hydro- chloride	0.02270	"	"	52.5	57.5	57.5	28.3
		0.01136	"	"	52.5	52.5	57.5	43.7
		0.00568	"	"	39.5	57.5	62.5	57.5
		0.00284	"	"	39.5	33.3	52.5	25.8
		0.00142	"	"	31.7	33.3	43.7	25.8
	Control	0	"	"	27.5	27.5	27.5	8.3

TABLE 11 (continued)

Bulls	Thia- mine deriva- tives	Concentra- tion of test com- pounds in the thawed semen	Viability index					
			Before the refri- gera- tion	After thawing				
				Just after thaw- ing	Just after the addi- tion	4 hrs. after the addi- tion	12 hrs. after the addi- tion	24 hrs. after the addi- tion
B	Thiamine propyl disul- fide	0.02270	"	"	57.5	62.5	52.5	44.2
		0.01136	"	"	57.5	62.5	57.7	52.9
		0.00568	"	"	57.5	57.5	57.7	52.9
		0.00284	"	"	52.9	57.5	52.9	52.9
		0.00142	"	"	52.9	52.9	44.2	40.0
	Thiamine propyl disul- fide hydro- chloride	0.02270	"	"	62.5	62.5	62.5	48.3
		0.01136	"	"	62.5	62.5	62.5	62.5
		0.00568	"	"	57.5	62.5	62.5	57.5
		0.00284	"	"	57.5	52.9	48.3	35.8
		0.00142	"	"	52.9	52.9	52.9	35.8
	Control	0	"	"	48.3	52.9	40.0	26.7

EXAMPLE 12

Bovine semen collected from 2 bulls is diluted 10-fold with an egg yolk-citrate buffer solution containing 2.4% of sodium citrate and 20% of egg yolk and the diluted semen are mixed with the thiamine derivatives described in the following Table 12 in a concentration of 0.01250%, 0.0625% or 0.0313%. Each of the diluted semen is stored at 4°C for 4 days. The "viability" and "motility" of the spermatozoa in the diluted semen is shown in the Table 12.

TABLE 12

Bulls	Thiamine derivatives	Concentration (%)	Storage period (days)				
			Just after the addition	1	2	3	4
	Thiamine propyl disulfide hydrochloride	0.01250	85+++	80+++ 5+	80+++ 5+	70+++ 10+	60+++ 10+
		0.00625	80+++ 5+	80+++ 5+	70+++ 5+	60+++ 5+	
		0.00313	80+++ 5+	75+++ 5+	70+++ 5+	60+++ 5+	55+++ 5+

TABLE 12 (continued)

Storage period (days)

TABLE 12 (continued)

Bulls	Thiamine derivatives	Concentration (%)	Storage period (days)			
			Just after the addition	1	2	3 4
A	S-benzoyl-thiamine O-mono-phosphate	0.01250	80+++	75+++	70+++>	65+++
			5+	5+	5+	5+
		0.00625	80+++	75+++	70+++	55+++
			5+	10+	5+	5+
		0.00313	80+++	75+++	65+++	50+++~
			5+	10+	5+	5+
	O,S-diacetyl-thiamine hydro-chloride	0.01250	80+++	70+++	65+++	40+++~
			5+	5+	5+	10+
		0.00625	80+++	75+++	65+++	45+++~
			5+	5+	5+	5+
		0.00313	80+++	75+++	60+++	40+++~
			5+	5+	5+	10+
	O,S-cyclo-carbo-thiamine hydro-chloride	0.01250	80+++	75+++	70+++	50+++
			5+	5+	5+	5+
		0.00625	80+++	75+++	65+++	40+++~
			5+	5+	5+	10+
		0.00313	80+++	75+++	55+++	40+++~
			5+	5+	5+	5+

TABLE 12 (continued)

Bulls	Thiamine derivatives	Concentration (%)	Storage period (days)			
			Just after addition	1	2	3 4
	TATD*	0.01250	80+++	80+++	75+++	70+++
			5+	5+	5+	5+
		0.00625	80+++	80+++	70+++	60+++
		0.00313	5+	5+	5+	10+
			80+++	75+++	65+++	55+++
			5+	5+	5+	5+
	bis-(O-benzoyl-thiamine) disulfide	0.01250	80+++	80+++	75+++	60+++
			5+	5+	5+	10+
		0.00625	80+++	75+++	65+++	60+++
		0.00313	5+	5+	5+	10+
			80+++	75+++	55+++	50+++
			5+	5+	10+	5+
	Thiamine 2-hydroxyethyl disulfide hydrochloride	0.01250	80+++	80+++	75+++	65+++
			5+	5+	5+	5+
		0.00625	80+++	80+++	70+++	65+++
		0.00313	5+	5+	10+	5+
			80+++	75+++	70+++	60+++
			5+	5+	5+	5+

TABLE 12 (continued)

Bulls	Thiamine derivatives	Concentration (%)	Storage period (days)				
			Just after the addition	1	2	3	4
	O,S-dibenzoyl-thiamine	0.01250	80++++	80++++	80++++	60++++	45++++~++++
			5+	5+	5+	10+	10+
		0.00625	80++++	80++++	70++++	60++++	50++++~++++
	O,S-dicarboethoxy thiamine hydrochloride	0.00313	5+	5+	10+	5+	5+
			80++++	75++++	70++++	60++++	50++++~++++
		0.01250	5+	5+	5+	5+	5+
		0.01250	80++++	80++++	80++++	70++++	45++++~++++
			5+	5+	5+	5+	10+
		0.00625	80++++	80++++	70++++	60++++	50++++~++++
	Control	0.00313	5+	5+	10+	5+	5+
			80++++	75++++	60++++	55++++	50++++~++++
		0	5+	5+	5+	5+	5+

0.00313	80++++	75++++	70++++	60++++	50++++~++++
	5+	5+	5+	5+	5+

TABLE 12 (continued)

Bulls	Thiamine derivatives	Concentration (%)	Storage period (days)				
			Just after the addition	1	2	3	4
	Thiamine propyl disulfide hydrochloride	0.01250	80+++	80+++	80+++	Not observed	20+++~+
			5+	5+	5+		10+
		0.00625	80+++	80+++	80+++	"	65+++
			5+	5+	5+		5+
	S-benzoyl-thiamine O-mono-phosphate	0.00313	80+++	75+++	70+++	"	50+++~+++
			5+	5+	5+		5+
		0.01250	80+++	80+++	75+++	"	45+++~+++
			5+	5+	5+		5+
		0.00625	80+++	80+++	70+++	"	55+++
		5+	5+	5+		5+	
	O,S-diacetyl-thiamine hydrochloride	0.00313	80+++	70+++	60+++	Not observed	45+++~+++
			5+	5+	5+		5+
		0.01250	80+++	70+++	60+++~+++	"	45+++~+++
			5+	10+	5		10+
		0.00625	80+++	75+++	70+++	"	40+++~+++
			5+	5+	5+		10+
		0.00313	80+++	70+++	60+++	"	40+++~+++
			5+	5+	10+		10+

0.00313	5+	5+	10+	”	10+	4.++++~+++
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TABLE 12 (continued)

Bulls	Thiamine derivatives	Concentration (%)	Storage period (days)			
			Just after the addition	1	2	3 4
B	O,S-cyclocarbo thiamine hydro-chloride	0.01250	80+++	75+++	70+++	40+++~+++
			5+	5+	5+	10+
		0.00625	80+++	75+++	70+++	40+++
			5+	5+	5+	10+
		0.00313	80+++	70+++	65+++	40+++~+++
			5+	10+	10+	10+
	TATD*	0.01250	80+++	80+++	80+++	60+++
			5+	5+	5+	5+
		0.00625	80+++	80+++	70+++	50+++
			5+	5+	5+	5+
		0.00313	80+++	70+++	60+++	40+++~+++
			5+	5+	10+	10+

TABLE 12 (continued)

Bulls	Thiamine derivatives	Concentration (%)	Storage period (days)			
			Just after the addition	1	2	3 4
	bis-O-benzoyl-thiamine)-disulfide	0.01250	80+++	80+++	75+++	50+++
			5+	5+	5+	5+
		0.00625	80+++	80+++	65+++	45+++~+++
			5+	5+	10+	5+
		0.00313	80+++	80+++	65+++	40+++~+++
			5+	5+	10+	10+
	Thiamine-2-hydroxyethyl disulfide hydrochloride	0.01250	80+++	80+++	75+++	30+++
			5+	5+	5+	10+
		0.00625	80+++	75+++	65+++	55+++
	Control		5+	5+	10+	5+
		0.00313	80+++	70+++	60+++	50+++~+++
			5+	5+	5+	5+
		0	80+++	70+++	60+++	45+++~+++
			5+	5+	10+	5+

TATD*: thiamine (7-methoxycarbonyl-3-acetylthioheptyl) disulfide

EXAMPLE 13

5 Sheep semen collected from 2 sires is diluted 10-fold with an egg yolk-citrate buffer solution containing 2.4% of sodium citrate, 20% of fresh yolk and 0.00625% of thiamine tetrahydrofurfuryl disulfide or its hydrochloride or thiamine propyl-
disulphide hydrochloride. Each of the diluted semen samples is stored at 4°C for
4 days. The viability and "motility" of the spermatozoa in the diluted semen samples
are shown in the following Table 13. 5

TABLE 13

Bulls	Thiamine derivatives	Concentration of test compounds in the semen diluter (%)	Storage period (days)				
			0	1	2	3	4
A	Control	0	80+++	75+++ 5+	60+++~+++ 5+	50+++~+++ 5+	30+++~+++ 10+
	Thiamine propyl disulfide hydrochloride	0.00625	80+++	80+++	75+++ 5+	70+++ 5+	60+++ 5+
	Thiamine tetrahydrofurfuryl disulfide hydrochloride	0.00625	80+++	80+++	70+++ 5+	70+++ 5+	60+++ 5+
B	Control	0	85+++	80+++ 5+	65+++~+++ 5+	50+++~+++ 5+	35+++~+++ 5+
	Thiamine propyl disulfide hydrochloride	0.00625	85+++	80+++ 5+	80+++ 5+	75+++ 10+	70+++ 5+
	Thiamine tetrahydrofurfuryl disulfide	0.00625	85+++	80+++ 5+	80+++ 5+	75+++ 10+	70+++ 5+

EXAMPLE 14

Sheep semen collected from 2 bulls is diluted five times with an egg yolk-citrate buffer solution (the first diluter) containing 2.4% of sodium citrate, 20% of fresh yolk and the resulting diluted semen is cooled to 4°C gradually for 1 hour. To the diluted semen is added egg yolk-citrate buffer solution (the second diluter) containing 2.4% of sodium citrate, 20% of fresh yolk, 14% of glycerin and 0.0125% of thiamine propyl disulfide hydrochloride or thiamine tetrahydrofurfuryl disulfide hydrochloride. After being kept standing at 4°C for 12 hours, 1 ml. each of the diluted semen samples is put into a plastic straw of 1 ml. capacity and then the straws are closed. After being frozen and stored in a dry-ice refrigerator at -79°C for 7 days, each of the semen samples in the straws is thawed at 4°C and stored at even temperature for 12 hours. The "viability" and "motility" of the respective thawed semen samples is shown in the following Table 14.

TABLE 14

Bulls	Thiamine derivative	Concentration of test compounds in the second diluter (%)	Before freezing	Just after thawing	12 hours after thawing
A	Control	0	80+++	35+++ 10+	10++
	Thiamine propyl disulfide hydrochloride	0.0125	80+++	65+++ 10+	45+++ 10+
	Thiamine tetrahydro-furfuryl disulfide hydrochloride	0.0125	80+++	65+++ 10+	45+++ 5+
B	Control	0	85+++	40+++ 5+	20+++~+
	Thiamine propyl disulfide hydrochloride	0.0125	85+++	75+++ 10+	45+++ 5+
	Thiamine tetrahydro-furfuryl disulfide hydrochloride	0.0125	85+++	75+++ 10+	45+++ 10+

EXAMPLE 15

Fertility test is conducted on the bovine semen preparation of the present invention. Bovine semen collected from 4 bulls is diluted 10-fold with an egg yolk-citrate buffer solution (containing 2.4% of sodium citrate and 20% of egg yolk) supplemented with 0.00625% of thiamine propyl disulfide hydrochloride. The diluted semen is inseminated to the female animals after being stored at 4°C for 1 to 5 days. The results are shown in the following table 15. In the results, percentage of conception comes from the first insemination.

TABLE 15

Bulls	Controls (not containing thiamine propyl disulfide hydrochloride)		Samples	
	Number of inseminated animals	Percentage of conception	Number of inseminated animals	Percentage of conception
A	1190	59.8	220	64.0
B	205	56.6	156	69.9
C	247	55.4	28	60.7
D	14	57.1	16	62.5

EXAMPLE 16

Fertility test is conducted on the bovine semen preparation of the present invention.

Bovine semen collected from 3 bulls is diluted 5-fold at the body temperature with an egg yolk-citrate buffer solution containing 2.4% of sodium citrate and 20% of fresh egg yolk, and the resulting diluted semen is cooled to 4°C gradually for 1 hour. The diluted semen is further diluted with an egg yolk-citrate buffer solution containing 2.4% of sodium citrate, 20% of fresh yolk, 14% of glycerin and 0.0125% of thiamine tetrahydrofurfuryl disulfide hydrochloride at 4°C. The diluted semen is kept standing at 4°C for 10 hours. 1 ml. each of the diluted semen samples is put into an ampoule of 2 ml. capacity and the ampoules are closed. After being frozen and stored in a liquid-nitrogen refrigerator at -180°C for 1 to 2 weeks, each of the ampouled semen preparations is thawed at 4°C, and subsequently inseminated to the female animals. The results are shown in the following table 16. In the results, percentage of conception comes from the first insemination.

TABLE 16

Bulls	Controls (not containing thiamine tetrahydrofurfuryl disulfide hydrochloride)			Samples	
	Number of inseminated animals	Percentage of conception	Number of inseminated animals	Percentage of conception	
A	181	49.2	35	68.2	
B	122	70.5	74	82.4	
C	86	56.9	147	64.6	

The terms "viability" and "motility" as used herein are referred to in the text-book by J.A. Laing entitled "Fertility and Infertility in the Domestic Animals" at pages 67 to 69 (1955), published by Bailliere, Tindall and Cox.

WHAT WE CLAIM IS:—

1. A stabilised semen preparation for artificial insemination of livestock, which is composed of livestock semen, a semen diluter and from 0.001 to 0.1 g per 100 c.c. of the total preparation of an unsymmetrical thiamine organic disulphide derivative (as herein defined) a thiamine derivative of the formula (II) (as herein defined) a bisthiamine-disulphide derivative (as herein defined), a salt thereof, or any mixture of these compounds.

2. A stabilised semen preparation for artificial insemination of ruminant livestock, which is composed of semen of ruminant livestock, a semen diluter and from 0.001 to 0.1 g per 100 c.c.s. of the total preparation of an unsymmetrical thiamine organic disulphide derivative (as herein defined), a thiamine derivative of the formula (II) (as herein defined), a bisthiamine-disulphide derivative (as herein defined), a salt thereof or any mixture of these compounds.

3. A stabilised semen preparation as claimed in claim 2, wherein the ruminant livestock is cattle, goats or sheep.

4. A stabilised semen preparation as claimed in any of claims 1 to 3, wherein the unsymmetrical thiamine organic disulphide derivative is thiamine propyl disulphide, thiamine tetrahydrofurfuryl disulphide, thiamine allyl disulphide, thiamine (7-methoxycarbonyl-3-acetylthioheptyl) disulphide, thiamine 2-hydroxyethyl disulphide, a salt thereof or any mixture of these compounds.

5. A stabilised semen preparation as claimed in any of claims 1 to 3 wherein the thiamine derivative of the formula (II) is O,S-diacetyl-thiamine, O,S-dibenzoyl-thiamine, S-acetyl-thiamine-O-monophosphate, O,S-dicarboethoxy thiamine, O,S-cyclocarbo-thiamine, a salt thereof, or any mixture of these compounds.

6. A stabilised semen preparation as claimed in any of claims 1 to 3 wherein the bisthiamine-disulphide derivative is bisthiamine-disulphide, bis-(O-benzoyl-thiamine)disulphide, a salt thereof, or any mixture thereof.

7. A stabilised semen preparation as claimed in any of claims 1 to 6, wherein the stabilised semen preparation contains a total of from 0.006 to 0.1 g per 100 c.c.s. of the thiamine compound or compounds of the total preparation.

8. A stabilised semen preparation as claimed in any of claims 1 to 7 wherein the semen diluter is an egg yolk diluter or a milk diluter.

9. A stabilised semen preparation as claimed in claim 8, wherein the semen diluter is an egg yolk-citrate buffer solution.

10. A stabilised semen preparation as claimed in claim 8, wherein the semen diluter is an egg yolk-phosphate buffer solution.

11. A stabilised semen preparation as claimed in claim 8, wherein the semen diluter is a milk diluter which is prepared by heating whole milk or skim milk.

12. A semen diluter for the artificial insemination of ruminant livestock, which contains from 0.001 to 0.1 g per 100 c.c.s. of the semen diluter of an unsymmetrical thiamine organic disulphide derivative (as herein defined), a thiamine derivative of formula (II) (as herein defined), a bis-thiamine-disulphide derivative (as herein defined), a salt thereof or any mixture of these compounds.

13. A semen diluter as claimed in claim 12 which is an egg yolk diluter or a milk diluter.

14. A semen diluter as claimed in claim 12 which is an egg yolk-citrate buffer solution.

15. A semen diluter as claimed in claim 12, which is an egg yolk-phosphate buffer solution.

16. A semen diluter as claimed in claim 12, which is a milk diluter prepared by heating whole milk or skim milk.

17. A semen diluter as claimed in any of claims 12 to 16 wherein the unsymmetrical thiamine organic disulphide derivative is thiamine propyl disulphide, thiamine tetrahydrofurfuryl disulphide, thiamine allyl disulphide, thiamine (7-methoxycarbonyl-3-acetylthioheptyl) disulphide, thiamine 2-hydroxyethyl disulphide, a salt thereof, or any mixture of these compounds.

18. A semen diluter as claimed in any of claims 12 to 16, wherein the thiamine derivative of formula (II) as herein defined is O,S-diacetyl-thiamine, O,S-dibenzoyl-thiamine, S-acetyl-thiamine O-monophosphate, O,S-dicarboethoxy thiamine, O,S-cyclocarbo thiamine a salt thereof or any mixture of these compounds.

19. A semen diluter as claimed in any of claims 12 to 16 wherein the bisthiamine-disulphide derivative is thiamine-disulphide, O-benzoylthiamine-disulphide a salt thereof, or any mixture of these compounds.



20. A semen diluter as claimed in any of claims 12 to 16, wherein the semen diluter contains from 0.006 to 0.01 g per 100 c.cs. of the thiamine compound or compounds of the semen diluter.

21. A method of increasing and/or maintaining the viability and motility of spermatozoa in a semen preparation for the artificial insemination of livestock, especially of ruminant livestock, containing semen and semen diluter which comprises incorporating into the preparation an unsymmetrical thiamine organic disulphide derivative (as herein defined), a thiamine derivative of formula (II) (as herein defined), a bithiamine-disulphide derivative (as herein defined), a salt thereof or any mixture of these compounds, in a concentration range of from 0.001 to 0.1 g per 100 c.c. of the total preparation.

22. A method according to claim 21, wherein the unsymmetrical thiamine organic disulphide derivative is thiamine propyl disulphide, thiamine tetrahydrofurfuryl disulphide, thiamine allyl disulphide, thiamine (7-methoxycarbonyl-3-acetylthioheptyl) disulphide, thiamine 2-hydroxyethyl disulphide, a salt thereof, or any mixture of these compounds.

23. A method according to claim 21 wherein the thiamine derivative of formula (II) is O,S-diacyl-thiamine, O,S-dibenzoyl-thiamine, S-acetyl-thiamine O-mono-phosphate, O,S-dicarboethoxy thiamine, O,S-cyclocarbo thiamine, a salt thereof or any mixture these compounds.

24. A method according to claim 21, wherein the bithiamine-disulphide derivative is thiamine-disulphide, O-benzoyl-thiamine-disulphide, a salt thereof, or any mixture of these compounds.

25. A method according to claim 21, wherein the total concentration range of the thiamine compound or compounds in the semen preparation is from 0.006 to 0.01 g per 100 c.c. of the preparation.

26. A stabilised semen preparation as claimed in claim 1, substantially as herein described.

27. A stabilised semen preparation as claimed in claim 1, substantially as herein described with reference to any of the specific examples.

28. A semen diluter as claimed in claim 12 substantially as herein described.

29. A semen diluter as claimed in claim 12 substantially as herein described with reference to any of the specific examples.

30. A method as claimed in claim 21 substantially as herein described.

31. A method as claimed in claim 21 substantially as herein described with reference to any of the specific examples.

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