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Effect of In-coherent Light on the Bacterial Contamination and Semen Quality of Semen Extended Media

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ABSTRACT

Semen extenders are used in artificial insemination process. These media can be infected by pathogens in different ways. Treatment of these media against pathogens claims enough integrity due to subsistence of semen. In this work a saffian method was applied to inhibit bacterial growth of semen media with minimal risk on the sperm viability. This work was aimed to investigate the effect of sunlight and solar simulator exposure times and doses as two different parameters that can be inspected for best semen quality and highest bacterial decontamination of semen media used for artificial insemination purposes. Applying different light sources gave an addational chance to clench this method indoor and outdoor treatment. The sunlight irradiances (427 & 526 W/m^2) are suitable for application at all of exposure times. For solar simulator exposures the most proper exposure time is 60 min for the irradiances 300 W/m^2 and 400 W/m^2 and all exposure times for the irradiance 500 W/m^2.

Key Words: Semen; Extended media; Bacteria; Light

INTRODUCTION

In cattle the first attempts of *in vitro* fertilization were already performed in the late of past century (Sreenan, 1970), but it was not successful until 1982, when the first calves were born after transfer of complete *in vitro* produced embryos (Brackett *et al*., 1982). Many attempts have been done to improve the multi-step process of *in vitro* production of embryos by refining the procedures of *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) of oocytes and *in vitro* culture of embryos (IVC). In 2001, worldwide 109.205 bovine embryos were produced *in vitro* (Thibier, 2002).

One of the main objectives of the artificial insemination (AI) industry is the improvement of livestock through the intensive use of genetically selection of highly potent sires. Therefore the semen of top bulls are highly demanded. The applications of artificial insemination in cattle in Egypt is becoming very important as it enables the use of selected sires for improving both milk and meat quality.

Artificial insemination offers one of the most effective means for distribution of high qualities genetic material of proven bull over a large number of females. For the success of AI technique it is necessary that used semen is free of microbial contamination (Kim & Son, 1998).

Early in the development of AI, it was recognized and established that many Pathogenic organisms could be bull semen companion and that the hazards to animal health using contaminated fresh or frozen semen were very great (Bielanski *et al*., 2003).

A number of well-written reviews have documented the coexistence of a variety of microorganisms in bull semen and the necessity to produce specific pathogen free bull semen (Bartlett, 1981; Hare, 1985).

Elimination of pathogens from bovine semen is primary concern of the artificial insemination (AI) industry and animal production. However, antibiotic resistant strains and a growing list of opportunistic and potentially pathogenic bacteria in semen are weakening the traditional reliance on vortexing, antibiotic and washing for bacterial control (Farca, 1997).

Bacterial contamination of *in vitro* and *in vivo* produced embryos presents a particular danger, because of the alteration of the zona pellucida and the use of various biological products during culture (Hiroshi *et al*., 1999; Guerin *et al*., 2000).

So new methods to supplement or replace antibiotic treatment in controlling semen contamination are required With the aim of producing pathogen-free semen, the present study was excuted. The objective of this work is to study whether sunlight and solar simulator exposure parameters Photo-stimulate the viability of spermatozoa and the bacterial count of contaminated cattle semen.

MATERIALS AND METHODS

Light exposure. Oriel Corporation Solar Simulator was

used for artificial light exposure. The fluence rate of artificial light was adjusted by change the value of electrical power of the xenon lamb (200 - 1200 W) and the light irradiance was measured by the Eldonet dosimeter, which was supplied by a software to determine the amount of visible light by W m^{-2} and UV-A, UV-B as well as temperature. Extended semen, which was packed in straw (0.25 mL), were irradiated inside cooling icebox to reduce the heat of xenon lamp. The UV portion of lamp was masked by using UV filter.

The light spot have been chosen for homogeneous illumination of the straws. Sunlight irradiances were measured as an average of incident light irradiances during exposure period.

Semen collection, evaluation and preparation. This work was carried out at the International Livestock Management Training Center (ILMTC) Sakha, kafer El- Shikh, Animal production Research Institute, Ministry of Agriculture.

Three sexually mature Friesian bulls were used in the present study. All bulls were healthy and clinically free from venereal diseases.

Semen was collected from the bulls by means of an artificial vagina. The temperature of artificial vagina was adjusted to be about 40 - 42°C. Minimum amount of sterilized glycerol was used for lubrication. The bulls were sexually stimulated before ejaculation.

Only semen samples of more than 80% mass motility were used throughout all experiments. Samples were selected to be of high density and vigraus progressive sperm motility with minimum % of dead and abnormal spermatozoa.

The tests of sperm motility were quickly done after light irradiation by placing a drop of semen on a warmed slide (37˚C) and then semen was examined under low power (x 10). The mass motility is scored from 0 - 100% after semen collection, ejaculates were placed in a water bath and were adjusted to 38˚C until evaluation and extention were completed.

Dead sperm percentage was carried out using eosin. The live sperms do not adopt eosin stain and therefore they are light in color, while the dead sperms absorb the stain to be colored. The concentration of eosin is 5% eosin in distilled water. The dead sperm percentage was done by placing small semen drop about twice the size of a pinhead on warmed slide a drop of eosin double as semen was placed on the slide. By means of a glass rod, the semen and eosin were carefully mixed and after a few seconds the mixture was smeared and examined under microscope. The differentiation between the live and dead sperm cells was clearly seen, since those cells, which were dead at the moment of mixing have taken up the eosin stain and showed up red color, whereas the cells, which were living remained un-colored.

Semen dilution. One extender was used in all experiments. Each 100 mL of the final extender consisted of 3.025 g. Tris, 1.675 g Citric acid, 0.75% glucose, 15% eggyolk, 7% glycerol, 100,000 µg streptopenclin, 100.000 i.u. penicillin, 0.25 g Lincospectin and double distilled water up to 100 mL. The extender is warmed up to 38˚C to be ready for use. In all experiments the final dilution rate was 1:20. The extended semen was cooled to 5ºC in a refrigerator over 2 - 3 h. Extended semen was packed in straw (0.25 mL) or in tubes for different experimental treatments.

Bacteriological counts. The types of bacteria, which contaminate the semen extender media were isolated and identified in the microbiology laboratory of vaccine institute-ministry of health, Egypt. All bacterial types were considered in this work as one group.

For the determination of viable bacterial count the medium was prepared by dissolving 23 g nutrient agar (Gilco lab USA) in 1000 mL of distilled water and broiled to boiling point. The solution was autoclaved at 121˚C under 15-lb/inch pressure for 20 min. Sterilized petri dishes have at least 85 mm inside diameter were used. Two straws (2*0.5 mL) were poured in each petri dish. The carefully closed petri dishes were incubated at 32ºC for 48 h. Manual counting were performed with the aid of magnification under uniform and properly controlled artificial illumination (American Public Health Association APHA, 1992).

All the colonies are counted including those of pinpoint size. Serial dilutions were prepared and viable numbers enumerated using pour plate technique. Replicates of four dishes for each treatment and control sample were performed.

Serial dilutions were prepared and viable numbers enumerated using pour plate technique. The results were expressed as CFU/mL fermented milk according to, American Public Health Association (APHA) (1992).

Statistical analysis. The raw semen data were statically analyzed by the methods of least square analysis of variance using the general linear model procedures of SAS (1987). Duncan multiple range test was used to test the differences among means (Duncan, 1955). Also T-test according to steel and Torrie (1980) was used to test significance of differences between two groups.

The percentage of sperm motility, live sperm, sperm abnormality and intact acrosome spermatozoa were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentage (Tables I - IV).

RESULTS AND DISCUSSION

Fig. 1 reveals the effect of different exposure times of 300 W/m^2 sun light irradiance on the bacterial count and the percentages of sperm motility and dead sperm %. It was found that the bacterial growth was reduced by 40% after 20 min exposure and this effect was stable until 60 min' exposure. For all exposure times of this experiment the percentages of sperm motility and dead % were within the

Table I. Analysis of variance for factors affecting bacterial count as a result of sun light treatment

Source of Variance	Degrees of freedom	Mean Squares	F value	P >
Season	3	8195.072	2771.62	0.0001**
Time of exposure (seconds)	5	68.490	23.16	0.0001**
Replicates of samples	3	5.585	1.89	0.1300ns
Residual	748	2.957	-	-

Table II. Analysis of variance for factors affecting bacterial count as a result of solar simulator (artificial light) treatment

Source of Variance	Degrees of freedom	Mean Squares	F value	P >
Dose of irradiation (Artificial light)	2	23.723	12.40	0.0001**
Time of exposure (seconds)	4	180.322	94.28	0.0001**
Replicates of samples	3	0.643	0.34	0.7993ns
Residual	515	1.912	-	-

Table III. Analysis of variance for factors affecting semen motility as a result of sun light treatment

Source of Variance	Degrees of freedom	Mean Squares	F value	P >
Season	3	0.1683	23.62	0.0001**
Time of exposure (seconds)	5	0.3850	54.03	0.0001**
Residual	157	0.0071	-	-

Table IV. Analysis of variance for factors affecting semen motility as a result of solar simulator (artificial light) treatment

Source of Variance	Degrees of freedom	Mean Squares	F value	P >
Dose of irradiation (Artificial light)	2	0.0520	3.93	0.0242*
Time of exposure (seconds)	4	0.2713	20.52	0.0001**
Residual	68	0.0132	-	-

P critical value
* Significantly different
** High significantly different
n.s Not significant

allowed limits of artificial insemination centers. The percentage of dead semen does not exceed 10% and the percentage of sperm motility does not reduce more than 20%. The minimum, allowed percentage of sperm motility used for AI is 30% and the maximum allowed percentage of dead sperm used for AI is 65% (International Livestock Management Training Center (ILMTC)). This means that the samples of semen, which exposed to 300 W/m^2 of sunlight most proper for AI but the percentage of bacterial contamination is still significantly high.

Using high irradiances of sunlight such as 427.7 and 526 W/m^2 (Fig. 2 & Fig. 3, respectively)a significant inhibition of bacterial growth (95 - 100% reduction of bacterial counts, $P < 0.05$) is achived. The percentage of dead sperm was 0% in all exposure times as shown in Fig. 2 and it was 18, 19, 23% for 20. 40 and 60 min exposure times, respectively in Fig.3. The percentage of sperm motility does not detract more than 20% after 20, 40 and 60 min exposure to 427.7 and 526 W/m^2 sunlight irradiances (Fig. 2 & Fig. 3, respectively). The results of Fig. 2 and Fig. 3 are better than the results of Fig. 1 due to the significant retrench of bacterial count ($P < 0.05$), which ameliorate the quality of semen for AI process success.

Fig. 1. Effect of different exposure times of 270 W/m^2 sunlight irradiance on the bacterial count and semen viability of semen extender media

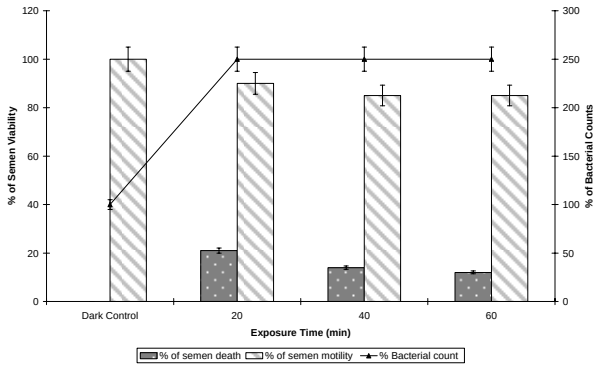


Fig. 2. Effect of different exposure times of 427 W/m^2 of sunlight irradiance on the bacterial count and semen viability of semen extender media

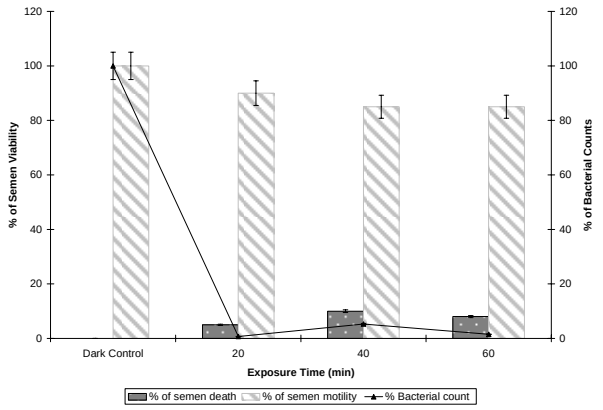
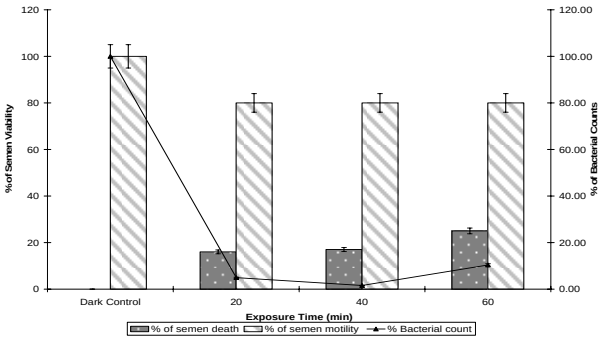


Fig. 3. Effect of different exposure times of 526 W/m^2 of sunlight irradiance on the bacterial count and semen viability of semen extender media



The samples of semen, which are subjected to sunlight of different environmental seasons, winter (300 W/m^2), spring (427.7 W/m^2) and early summer (527 W/m^2) reveale different impacts on bacterial growth. The best impact is got after spring and summer sunlight irradiances exposure. This means that sunlight is an efficient light source for this application in specific seasons of year but the AI could be done all over the year. This embolden of applying artificial light source, which can be controlled to give different irradiances having similar sun light spectrum. Solar simulator instrument was the most fit light source that embrace most of sunlight spectrum. This device can be controlled by tunable power supply to produce different light irradiances analogous to sunlight irradiances of different seasons.

The effect of solar simulator irradiances were slightly disparate from sunlight irradiances on the bacterial growth of extended semen medium. Fig. 4, 5 and 6 show the affection of 300, 400 and 500 W/m^2 irradiances of solar simulator, respectively on semenviability and bacterial growth of extended semen media.

300 W/m^2 (Fig. 4) minimize bacterial growth by 63, 72 and 95% in 20, 40 and 60 min light exposure, respectively. The percentages of sperm motility after such exposure times (90, 70 & 70, respectively) were within the allowed limits of AI centers. For the same exposure times the percentages of dead sperm were 10, 27 and 29, respectively. They are desired for AI. The best result in this experiment is present after the exposure time of 60 min, which induces the highest reduction of bacterial count and pat sperm motility and dead sperm percentage, so this sample under these conditions is favorable for AI.

Fig. 5 representes different demeanors of bacterial growth according to light exposure times of 20, 40 and 60 min. The bacterial growth increases at 20 and 40 min light exposure. 60 min exposure times causes 98% of bacterial reduction (*Staphilococcus aurieus*, catalase positive bacteria was added to the normal medium). The sperm motility of the same specimen does not encroach AI requirement and the disparity of its percetanges in variance exposure times are not significant. The percentages of dead sperm were within the AI demands too.

Fig. 6 reveals the effect of different exposure times of 500 W/m^2 solar simulator light on the bacterial count and percentage of semen motility and death. These results are nearly similar to the results of Fig. 3 due to their close irradiances similarity.

Two different parameters can induce bacterial inhibition under our experimental conditions. The thermal effect parameter, which agree with the previous work (David *et al*., 2005; Fumihito *et al*., 2005; Hilde *et al*., 2005) and the photochemical impacts due to visible bands of light sources.

Regarding to the previous work, the most effective band of visible light is the red color band. Living cell mitochondria can absorb red light by the components of the respiratory chain (i.e. Flavines & cytochromes), which causes an activation of cell respiration and the oxidation of

Fig. 4. Effect of different exposure times of 300 W/m^2 of solar simulator irradiance on the bacterial count and semen viability of semen extender media

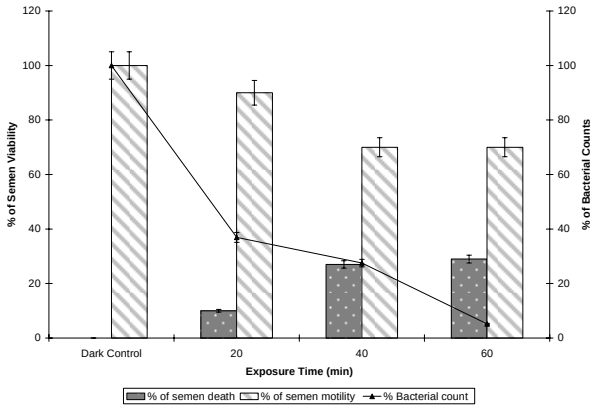


Fig. 5. Effect of different exposure times of 400 W/m^2 of solar simulator irradiance on the bacterial count and semen viability of semen extender media

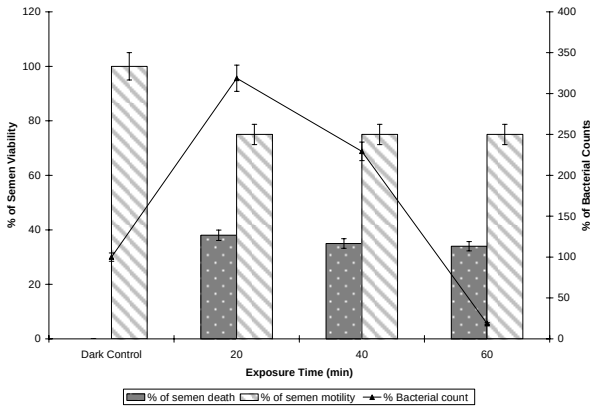
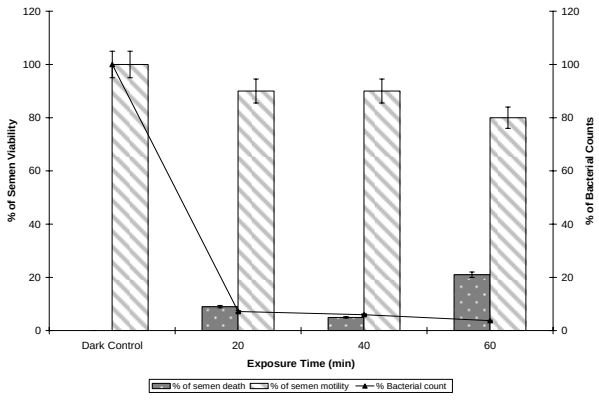


Fig. 6. Effect of different exposure times of 500 W/m^2 of solar simulator irradiance on the bacterial count and semen viability of semen extender media



the NAD pool. This may lead to the redox status of both mitochondria and the cytoplasm (Rachel *et al*., 1997; Ocana, 1997). This may interpret the improvement semen viability during and after light irradiation. Increase of cellular respiration is related to energy supply which increase semen motility.

It is well known (Natalia *et al*., 1998) that red light is also associated with stimulation of reactive oxygen species (ROS) generation, probably H_2O_2. The catalase negative bacteria have no ability to break down H_2O_2, which lead to bacterial death by its toxic effect. Semen also lack catalase activity (Yanagimachi, 1994) but they posses two alternate enzymatic defense systems (Super oxide dismutase & glutathione peroxidase/reductase pair) against dioxygen species $O^{-}_{2.}$ and H_2O_2 accumulation. This may interpret the red light fetal effect of bacteria not semen in a sample containing both semen and catalase negative bacteria, which exposed to light sources include the red light spectrum.

Catalase negative clean semen extnded media was used in the experiments of Fig. 5. This medium was provided by catalase positive bacteria (*Staphilococcus aurieus*), which are not affected by red light and this was done to confirm the reason of bacterial reduction is due to ROS production as a result of light irradiation. The result of Fig. 5 reveal bacterial growth enhancement at exposure times 20 and 40 min, which means that the increase of oxygen production due to break down of H_2O_2 activat the bacterial growth. At 60 min exposure time, a drop of bacterial growth happens. This may related to high production of hydrogen peroxide after light exposure in concentration higher than catalasis rate of H_2O_2 break down.

The difference in the efficiency between sunlight and solar simulator exposure for bacterial inhibition may be related to the light quality of two sources. This difference was discussed well in other previous work (El-Tayeb, 2000).

CONCLUSION

In the present work sunlight (costless light source) and solar simulator have been exploited to inhibit bacterial growth in extended semen. Although the use of sun light revealed relatively good results concerning bacterial growth and sperm motility and dead, but the seasonal nature of the sunlight made it necessary to substitute it by artificial light source (solar simulator). Exposure of samples to solar simulator light for different times reveales reasonable results, which can be very useful for field applications.Light source, light dose and time of exposure are three parameters have to be adjusted to proper values to have the desired effects of this technique.

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