Biosciences, Biotechnology Research Asia Vol. **4**(2), 777-780 (2007)

Ultrasonic studies on the different veterinary samples

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(Received: July 24, 2007; Accepted: September 01, 2007)

ABSTRACT

Ultrasonic velocity (v), attenuation coefficient (α), density (ρ), post thaw-motility, sperm concentration (n) and optical density for various bull semen samples of Deoni, Killeri, Jersey, Sruti, Holstein Friesian (HF) and Murrah were measured at room temperature of about 301.15 K temperature. Ultrasonic velocity (v) studies were carried out at the frequency of 2MHz with PZT transducer of X-cut using ultrasonic Pulse Echo Overlap (PEO) technique on various bull semen samples. The density (ρ) of various bull semen samples were measured with 10ml specific gravity bottle, the sperm concentration (n) by Heamocytometer, optical density by photoelectric colorimeter and post-thaw motility by Phase-Contrast Microscope. It was observed that the ultrasonic velocity (v) was different for different bull semen samples and relative merits were discussed in the present study.

Key words: Ultrasonic Velocity (v), Attenuation Coefficient (α), Sperm Concentration (n) and Pulse Echo Overlap (PEO) Technique

INTRODUCTION

Ultrasonic study is a useful technique for understanding the properties of the liquids. Many researchers[1-7] have studied the ultrasonic properties of liquids and biological cultures. There was no sufficient literature available on the study of ultrasonic behavior of bull semen samples. An attempt was made to study the ultrasonic properties on bull semen samples.

The bull semen contains certain sperm concentration and a thorough study of ultrasonic properties was aimed in the present study in addition to the optical density and post thaw measurements. In view of the extensive applications of ultrasound in medical diagnostics, a systematic study of ultrasonic behavior of bull semen of Deoni, Killeri, Jersey, Sruti, Holstein Friesian (HF) and Murrah were carried out. The density of bull semen samples varies from bull to bull and within the bull[8-12] depends upon the sperm concentration of the bulls. The results thus obtained were presented and discussed.

EXPERIMENTAL

The samples taken for present study were obtained from Veterinary College, Bider, Karnataka, India. The samples were thoroughly prepared with 1:1 ratio of sperm and PBS with the help of measuring suckers.

The ultrasonic velocity (v) and attenuation coefficient (α) measurements were made with the help of microprocessor based ultrasonic Pulse Echo Overlap (PEO) technique (model UX 4400M) with built in time, velocity and thickness measuring feature, with a frequency of 2MHz supplied by Roop Telesonic ultrasonic Ltd, Bombay, India. The internal circuit of ULTRASONIX 4400M was the fast switching solid state version and IC chips, for stability and accuracy.

It has a special memory feature of permanent storage and direct digital read out of velocity (v) and attenuation coefficient (α) of the samples. The accuracy of instrument in measuring velocity is ±1m/s and the accuracy of instrument in measuring attenuation coefficient is ± 3 dB.

The liquid sample was taken into the liquid cell by using a 10ml pipette with an accurate measurement. In the commonly used Pulse Echo Overlap (PEO) technique, ultrasonic waves are introduced into the sample through a piezo-electric transducer. The ultrasonic pulse travels through the sample and an echo is registered each time when it returns to the transducer. Now the corresponding longitudinal wave velocity (v) of ultrasound v = 2l/T in the given liquid medium is selected and displayed on the 8 digit counter and attenuation on two digit counter. Where v is the ultrasonic sound velocity (m/s), l- is the length of the liquid (sample) column (mm). T is the travel time between two selected consecutive echoes (μs).

The densities (ρ) of all the mixtures were determined with 10ml specific gravity bottle and mass (m) of a given volume of the liquid is determined by using a single pan electrical balance. The results of the densities were accurate to ± 0.5%.

Concentration of sperm was measured by using Heamocytometer. Heamocytometer is used in the direct cell count method. A drop of undiluted semen is placed in clean watch glass and added a very little speck of eosin powder just to stain the sperm to a pale pink colour. Stained semen is drawn up to 0.5mark in to a R.B.C pipette. Care was taken not to over fill or under fills the 0.5 mark. The tip of R.B.C pipette was removed and wiped it to become dry. In R.B.C pipette 1% formal saline was taken into the pipette up to 101mark exactly to reach the standard dilution rate of 1:200. The main function of formal saline is killing and dispersing the cell evenly through out the fluid. The pipette was vigorously shacked with quick wrist motion to ensure proper distribution of sperm.

The R.B.C counting chamber of the Heamocytometer was focused under the high power objective of the microscope. A cover slip was placed over the counting chamber. R.B.C pipette was taken and mixed once again, discarded the initial 3-4 drops and gently change in to the counting chamber, by placing a drop of the diluted semen near the cover slip. The semen was drowning in to the counting chamber due to capillary suction. The total numbers of sperm heads are counted after attaining uniform dispersion.

Total number of sperms counted = n
Concentration of sperm in one milliliter of semen = Volume correction factor x dilution correction factor x 1000 x n.

Post-thaw motility was estimated by using Phase-Contrast Microscope. The ends of the straw were cutes and collected the semen in a small test tube. A very small drop on clean slide was kept and covered it with a cover slip and examined under the phase-contrast microscope. Motility based on progressive motile sperm is estimated on the nearest 5 percent. If motility differs by 10 percent or more between two straws of the same batch, a third straw is examined.

40% post –thaw motility was adopted as the minimum acceptable motility for cattle and buffalo frozen semen in the present investigation studies. In routine practices only post thaw motility test is carried out and it is a reliable test when carefully done by the experienced personae. The remaining three tests (post-thaw acrosomal integrity, post-thaw release of acrosomal enzymes, post-thaw incubation survival of spermatozoa) are only as added tests when it is suspected that semen may be of lowered fertility but meets minimum standard for motility. Optical density was measured for different bull semen samples by using Photo-Colorimeter.

RESULTS AND DISCUSSION

Ultrasonic velocity (v) and attenuation coefficient (α) were recorded experimentally for different semen samples. The experimental values of ultrasonic velocity (v), attenuation coefficient (α) and density (ρ) were reported in Table.1. The experimental values of post-thaw motility, sperm concentration, and optical density were reported in Table.2. But there was an ambitious trend to know the relation between optical density and attenuation coefficient (α) of the samples. The absorption of sound energy (attenuation) and the absorption of

light energy (optical density) through the samples were similar in trend of the curve, shown in Fig .3 and Fig.6. Ultrasonic velocity and densities were shown in Fig.1 and Fig. 2. Post-thaw motility, sperm concentrations were shown in Fig. 4 and Fig.5. Ultrasonic velocity (v) and density (ρ) of Murrah were observed as high than the other sample. Ultrasonic velocity (v) and density (ρ) of all the samples are holds good in agreement with the sperm concentration.

Table 1: Ultrasonic velocity (v), attenuation coefficient (α), density (ρ) of various bull semen samples

Typesof bull semen sample	Ultrasonic velocity (v) m/s	Attenuation cofficeint(α) (dB)	Density (ρ)10^3 Kg/m^3
Deoni	1565	66	0.8856
Jersey	1555	64	0.8768
Killari	1551	63	0.8678
HF	1560	65	0.8778
Sruti	1545	63	0.8628
Murrah	1586	62	0.8884

Table 2: Post-thaw motility, sperm concentration, optical density of various bull semen sample

Typesof bull semen sample	Post-thaw molidity	Sprem Concentration	Optical density
Deoni	10%	1500 × 10^6ml	1.87
Jersey	30%	350 × 10^6ml	1.54
Killari	30%	150 × 10^6ml	1.36
HF	50%	900 × 10^6ml	1.59
Sruti	20%	200 × 10^6ml	1.39
Murrah	40%	4500 × 10^6ml	1.20

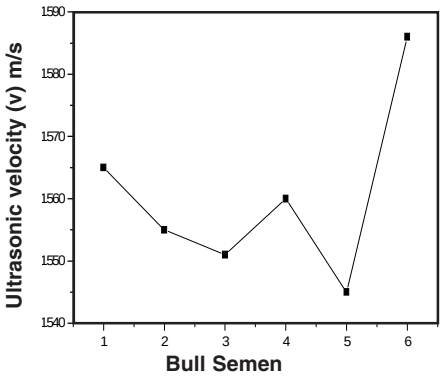

Fig. 1: Bull semen vs Ultrasonic velocity (v)

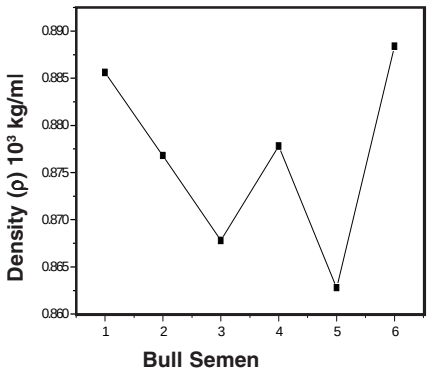

Fig. 2: Bull semen vs Density (ρ)

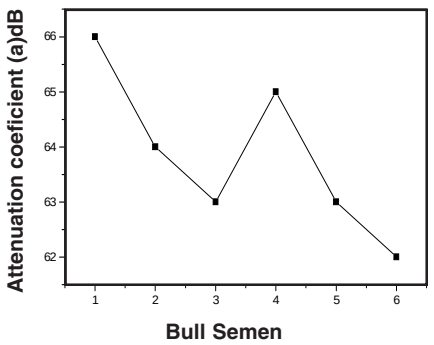

Fig. 3: Bull semen vs Attenuation coefficient (α)

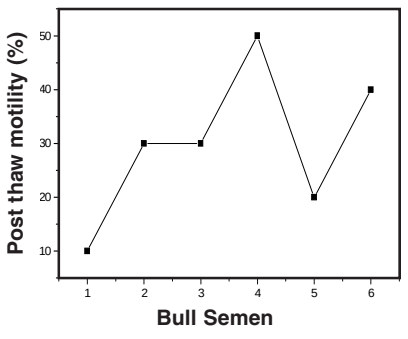

Fig. 4: Bull semen vs Post thaw motility

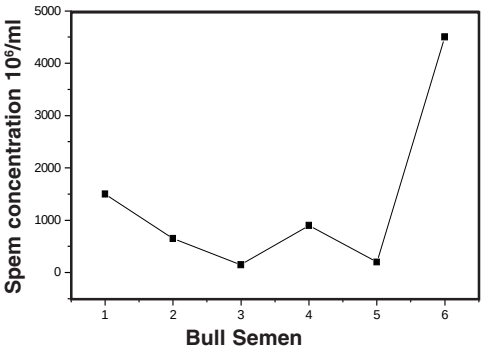

Fig. 5: Bull semen vs Sprem concentration

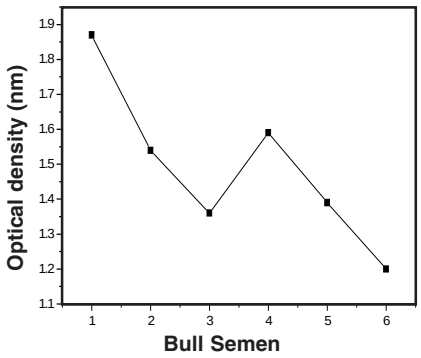

Fig. 6: Bull semen vs Optical density

CONCLUSION

Ultrasonic Investigation studies were carried out on various bull semen samples. It was found that the ultrasonic velocity (v) and attenuation coefficient (α) were different for different bull semen samples. But the densities and sperm concentrations were in similar trend. Ultrasonic velocity (v) and density are in good agreement with the sperm concentration.

ACKNOWLEDGMENTS

One of the authors (KSR) is thankful to Head, Department of Physics, Osmania University for encouragement and also thankful to Management, Principal and Head, Dept of Physics, S. P. College, for permitting to pursue my research. The author is also thankful to Professor S. Venkateshwar, Principal, Univ. College of Technology, Osmania University, Hyderabad, for valuable suggestions.

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