EFFECTS OF GLYCEROL AND FRUCTOSE ON LIVABILITY, 
MOTILITY, AND ANAEROBIC GAS PRODUCTION 
OF BOVINE SPERMATOZOA

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SUMMARY

In vitro manometric determinations of gas production by bovine spermatozoa under anaerobic conditions indicated a slight but statistically nonsignificant inhibitory effect of glycerol. The inclusion of 1% fructose with 1.0, 3.3, or 10.0% glycerol caused an immediate and marked stimulation of gas production by the spermatozoa. Glycerol plus fructose maintained a greater degree of motility and livability of bovine spermatozoa at the end of 105 min. of incubation at 38 °C. than did either fructose or glycerol alone. Under the conditions of this study, 1% glycerol plus 1% fructose was the apparent optimum for maintenance of spermatozoan motility and livability.

In 1949, Polge et al. (23) discovered that glycerol, a trihydroxy alcohol, would protect fowl spermatozoa during freezing and thawing. Since then, the mode of action (11, 12, 14, 25) and rate of entry (10, 13) of glycerol have been studied for several types of tissues. Although the practical aspects of freezing bovine spermatozoa have received considerable attention, relatively little work has been done on possible localization and mode of action of glycerol in these cells. Proof of entry has been presented largely by metabolic studies. Mann and White (16) found that glycerol was utilized aerobically but not anaerobically by ram spermatozoa. O'Dell et al. (21) observed that glycerol-1-C\textsuperscript{14} was utilized aerobically by bovine spermatozoa and converted, in part, to radioactive carbon dioxide. White et al. (27) found that glycerol, under the conditions of their study, increased the oxygen consumption of bovine spermatozoa. Pickett and Merilan (22) employed an autoradiographic technique in an attempt to determine the loci of glycerol action and found glycerol-1-C\textsuperscript{14} to be associated with all parts of the spermatozoa. However, more of the C\textsuperscript{14} appeared to be with the nuclear area of the head than with the other parts.

The investigations of Mann (15) have shown fructose to be the normal glycolyzable carbohydrate present in bovine semen and that fructose is broken down, in part at least, to lactic acid. It has been further found that the rate of glycolysis is greater in nitrogen than in air (2, 3, 15). This study was conducted in an attempt to provide additional information concerning the metabolism of various concentrations of glycerol and glycerol-fructose combinations under anaerobic conditions.

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MATERIALS AND METHODS

Bull semen was collected by means of an artificial vagina from five bulls maintained as part of the University of Missouri Agricultural Experiment Station Dairy Herd. Semen motility was rated according to the method of Herman and Madden (8) and only samples of four motility or better were used in the experiments. The spermatozoa were washed in calcium-free Krebs-Ringer phosphate (KRP), prepared according to Umbreit et al. (26), by suspending the semen in 167.0% of its original volume, centrifuging for 10 min. at 600 g, and removing the same amount of material that was added. Sperm cell concentration for each washed suspension was determined by the hemocytometer method (8).

Anaerobic metabolism of the spermatozoa was measured by gas production from the substrate, with KRP as the buffer. The measurements were made at 38°C in a rectangular constant-volume Warburg Respirometer accommodating 14 reaction vessels, and adjusted to a shaking speed of 114 strokes per minute. To each of the flasks was added 2.0 ml KRP, 0.5 ml. of the washed sperm suspension, and 0.5 ml of the substrate (glycerol, fructose, or glycerol-fructose) was placed in the side arm. Final volume in each flask was 3.0 ml. The flasks were gassed with a mixture of 95.0% nitrogen and 5.0% carbon dioxide, using a manifold gassing arrangement.

After placing the flasks in the 38°C water bath, they were allowed to equilibrate for 10 min. Following equilibration, readings were taken every 5 min. for 30 min., to establish a base. The side arms were then tipped and readings taken at 5-min. intervals for 75 min.

At the end of the experimental run, motility and per cent alive were determined on each flask. The per cent alive was calculated as the average of duplicate determinations made according to the method proposed by Mayer et al. (17).

RESULTS AND DISCUSSION

The data presented in Figure 1 indicate that gas production by bovine spermatozoa was slightly inhibited (not statistically significant at 5% level) by all concentrations of glycerol studied. The control flasks contained some residual fructose, since the washing procedure used in these studies was not designed to remove all the fructose present in the seminal plasma but merely to dilute it while keeping the spermatozoan concentration high. Therefore, the controls in this study probably utilized the remaining fructose, whereas in the flasks containing glycerol, the glycerol in some way interfered with fructose utilization. However, the inhibition was not directly proportional to the final concentration of glycerol present. In the flasks containing 8.3 and 10.0% glycerol, most of the inhibition occurred during the first 15–30 min. after tipping; whereas, at lower glycerol concentrations, less inhibition occurred in the same time interval. This indicated that perhaps glycerol concentrations of 7–10% should be added stepwise, as done by some investigators (1, 5, 6, 20, 24).
The addition of 1.0% fructose to the flasks, at the same time 1.0, 3.3, and 10.0% glycerol were added, caused an immediate and marked stimulation of gas production. However, these results do not show whether the increased gas production was the result of the utilization of fructose or glycerol, or both. In view of some recent investigations, it appears probable that the carbon dioxide was being produced from both glycerol and fructose. Mann (15) and Moore (19) have shown that glucose and fructose appear to be utilized through essentially identical pathways. Flipse and Alquist (4) have shown that lactic acid produced from glucose-C\textsuperscript{14} by bovine spermatozoa was, in part, broken down to carbon dioxide. O’Dell \textit{et al.} (21) found that glycerol-1-C\textsuperscript{14} was utilized by bovine spermatozoa under anaerobic conditions and converted in measurable amounts to radioactive carbon dioxide. An alternate possibility is that fructose might accelerate the entry of glycerol into the cell, as has been postulated by White \textit{et al.} (27) for arabinose, a nonglycolyzable pentose.

After 105 min. of incubation, motility and per cent alive determinations were made on each flask shown in Figure 1. The data taken from this study...
are presented in Figure 2. Mixner and Saroff (18) reported that glycerol levels above 4% in diluters containing egg yolk, skim milk, or whole milk interfered markedly with the dead-alive stain; however, that effect was not noted in preliminary trials for these studies, where the spermatozoa were suspended in KRP salt solutions.

The results of the motility and per cent alive studies show that in flasks containing glycerol without fructose, motility and per cent alive were lower than in the corresponding glycerol-fructose flasks. The only exception was seen in the per cent alive studies with 10% glycerol. The most desirable motility rating was obtained with the samples containing 1% glycerol plus 1% fructose. Since more gas was produced in the 10% glycerol-fructose flasks, motility may have been depressed, due to a build-up of metabolites toxic to the cells instead of a direct toxic effect produced by the high concentration of glycerol. However, all flasks containing glycerol alone showed a lower motility, indicating a depression which probably was not associated with a build-up of toxic substances, since less metabolic activity, as measured by gas production, was observed in
these flasks than in the controls which exhibited a higher motility. This difference in motility between spermatozoa suspended in glycerol and glycerol-fructose might, in part, be explained by the report of Kampschmidt et al. (9), which suggests that as glucose is used to replace the buffer solution, viability of bovine spermatozoa is increased. This effect was attributed to: 1) more glycolyzable sugar present, or 2) decreased electrolyte content of the storage medium. Further support for the electrolyte view has been presented by Hendrikse et al. (7). They found that glycerol exerted a considerable apparent osmotic effect, with a lowering of the freezing point, when freezing bovine spermatozoa for use in artificial insemination.

One per cent glycerol in the presence of 1% fructose appeared to be more beneficial to the sperm cells, both from a motility and per cent alive standpoint, than any other combination studied. Since a rather large amount of metabolic activity was observed in these flasks without the decrease in per cent alive and motility, as observed in the 10% glycerol-fructose flasks, perhaps low concentrations of glycerol, in the presence of fructose, can be converted largely to carbon dioxide without the accompanying increase in toxic metabolic substances. Thus, a beneficial rather than a depressing effect was exerted on the motility and viability of the cells.

REFERENCES


