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SHORT-TERM PRESERVATION OF SPERM UNDER OXYGEN ATMOSPHERE IN RAINBOW TROUT (*SALMO GAIRDNERI*)

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ABSTRACT

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Experiments were carried out to test the possibilities of using plastic bags inflated with oxygen to preserve and to transport rainbow trout sperm. Sperm survival is improved after storage in an O₂ atmosphere in comparison with storage in air. After storage for 5 days (Exp. 1) fertilizing ability was similar under O₂ to that of the controls (freshly collected sperm) but under air it was significantly lower ($P < 0.001$). In another experiment (Exp. 2), fertilizing capacity depended on the rate of dilution and the quality of the eggs. With some females high levels of fertilization were recorded after 14 days of sperm storage and with other females fertilizing capacity of the same sperm drops within one day. This variability in the response still limits the practical application of short-term preservation of rainbow trout sperm.

INTRODUCTION

Short-term preservation of gametes in liquid state has been studied intensively in salmonids. It is now well established that sperm kept undiluted at 4°C or around 0°C survive one or sometimes several days (Scheuring, 1924; Barrett, 1951; Dorier, 1951; Ginsburg, 1968; Takano et al., 1973; Carpentier and Billard, 1978; Hiroi, 1978). At a higher temperature (10°C) sperm survive for only a few hours (Withler and Morley, 1968; Billard, 1978). Factors other than temperature may influence sperm survival; for instance, a high air/sperm ratio (Truscott et al., 1968) or a supply of oxygen (Holtz et al., 1979; Stoss et al., 1978; Billard and Legendre, 1980). However, Foerster (1965) indicated that a large volume of air was unfavorable after 25 h of sperm storage.

In the present experiments survival of rainbow trout sperm after storage in O₂ or in air was compared. Experiments were carried out in polyethylene bags which are of practical interest for transportation of sperm.

MATERIAL AND METHODS

Brood stock (KA 76 A strain, 3 year old) was kept from hatching in the environment provided by our laboratory rearing facilities: recycled water

(Petit, 1974), temperature ranging from 6 to 21°C, and natural photoperiod. Experiments were carried out in mid January 1979, the rearing temperature being 9–10°C. Full spermiation (production of more than 0.5 ml of sperm after a single sampling) had started in mid December. Eggs used to test the fertilizing capacity of the sperm were taken from freshly ovulated females (less than one week).

Experiment 1

Sperm was preserved for 5 days. Two ml of sperm were taken individually from 10 males, pooled, mixed and poured into two polyethylene bags (0.5 l capacity each). Oxygen was blown into one bag and air into the other for 30 s. The bags were closed and stored at 4°C in a refrigerator in complete darkness. The bags were placed on their sides so that the maximum level of sperm never exceeded 0.5 cm.

Experiment 2

Sperm was stored under the same conditions as in experiment 1, but larger amounts were used (60 ml in each bag) from a larger group (20 males). Fertilizing ability was followed for 20 days by periodical sampling. After each sampling, done with a sterile Pasteur pipette, air and oxygen were blown into the bags for 10 s. Hence, the sperm samples in both bags were agitated during this procedure. Fertilizing capacity was tested, not in pooled eggs, as in experiment 1, but in lots of around 200 eggs taken individually at each sampling, from a group of seven females. At the end of the experiment (day 20), aliquots of eggs from each of the remaining females (6) were pooled and fertilized with freshly collected sperm taken from the initial group of 20 males; this served as a control.

For both experiments artificial insemination was performed according to Billard (1977); groups of about 150 eggs were used with 10 ml of diluent for insemination and 100, 10 or 1 μ l of sperm (dilution rates of 10^{-2} , 10^{-3} , 10^{-4} respectively). Eggs were incubated for 25 days in a recycled water system, thermoregulated at $10 \pm 1^\circ\text{C}$ and fixed in Stockard fluid. Embryonated eggs (normal, abnormal or aborted) were counted as well as non-fertilized eggs. In fact, aborted or abnormal embryos were very few, and fertilizing capacity of the sperm was estimated from the percentage of embryonated eggs (normal + abnormal + aborted). Variability was very high especially in experiment 2 and results are given individually for each female.

Statistical comparisons were made with the t test and variance analysis after angular transformation of the percentage.

RESULTS

Results of experiment 1 are given in Fig. 1. After 5 days storage in the O₂ atmosphere, fertilizing capacity of the sperm remained at the same level as

freshly collected sperm for each dilution tested. At 10^{-4} dilution the percentage of fertilization was slightly lower with oxygen-preserved sperm but the difference was not significant. In air, results are constantly and significantly ($P < 0.001$) lower in comparison with fresh and oxygen-preserved sperm.

In experiment 2 (Fig. 2) sperm survival varied dramatically when tested on batches of eggs taken from different females. Differences were not obvious between O_2 and air exposure at the 10^{-2} dilution rate (not significant with variance analysis) but they became noticeable after 8 days of preservation when the rate of dilution was increased. However, this pooled sperm retained its initial fertilizing capacity for 8 days with the eggs of only two females (and 14 days in the case of one female at 10^{-2} dilution). With two females the sperm fertilizing capacity dropped very quickly within 1 or 2 days. It should be noticed that the females which showed a rapid decrease of fertilizing capacity of the sperm had the lowest initial fertility. The final control at 20 days showed that the eggs were still quite fertile at that time: 80% at 10^{-2} and 71% at 10^{-3} .

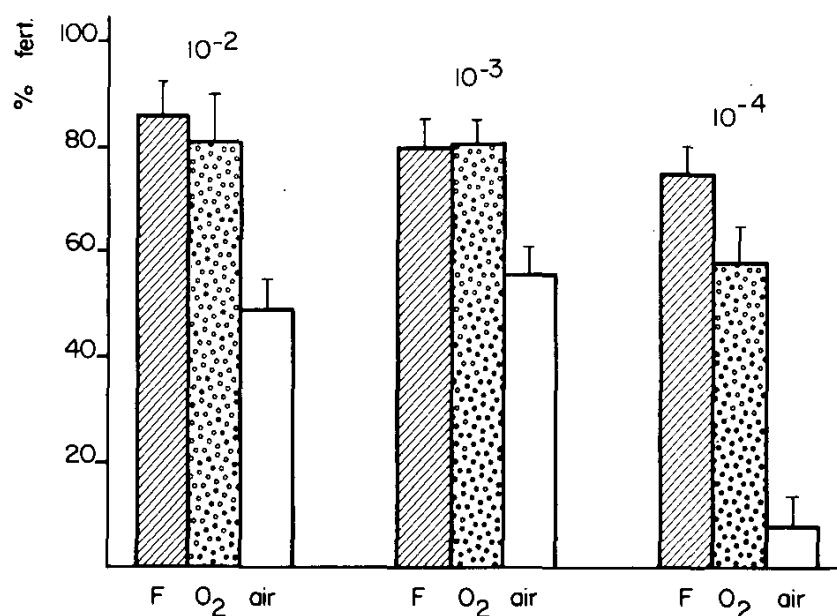


Fig. 1. Fertilizing capacity of rainbow trout undiluted sperm stored for 5 days in plastic bags inflated with oxygen (O_2) or air, compared with controls: freshly collected sperm (F). 10^{-2} to 10^{-4} : rate of dilution of sperm used for insemination.

DISCUSSION

These experiments show that sperm survival is improved after storage in oxygen but fertilization results depend on the quality of the eggs and the rate of dilution. When compared with storage in air, the superiority of O_2 appears after 5 days of preservation in experiment 1 and after 8–14 days in experiment 2. Fertility of gametes kept in vivo remained quite stable during the 20-day experiment, showing that sperm stored in air or in O_2 loses its fertilizing capacity more rapidly than in vivo. Therefore environment in the genital tract or the testis is more favorable than in vitro even if O_2 is provided.

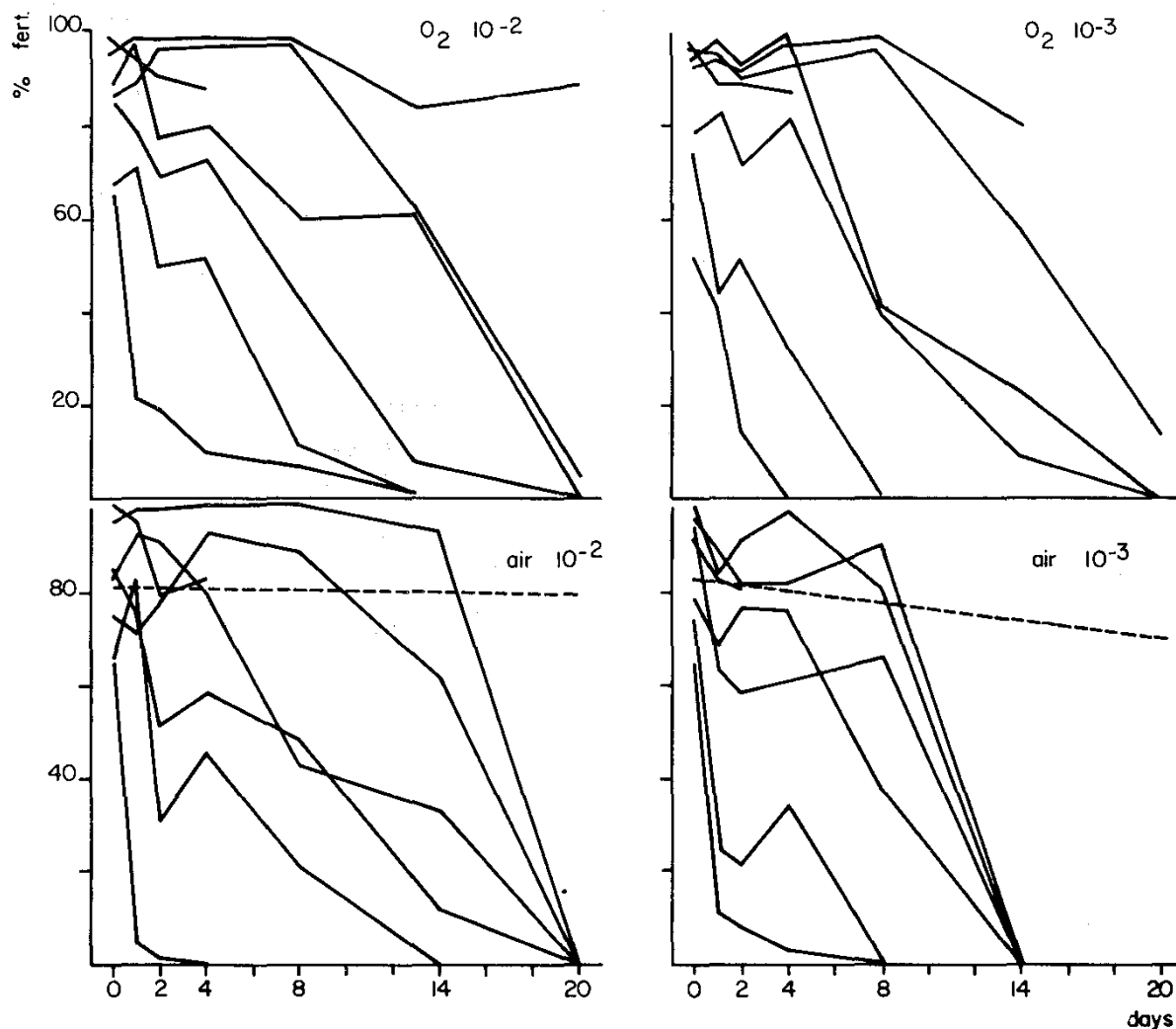


Fig. 2. Change of fertilizing capacity of rainbow trout sperm stored in plastic bags inflated with oxygen (O_2) or air during an experimental period of 20 days. Tests were carried out on aliquots of eggs taken periodically and individually from seven females. The dashed line (controls) indicates the average fertility of eggs (fertilized with fresh sperm).

Possibly the supply of O_2 is more homogeneous in vivo than in vitro. When sperm is left in the vas deferens of a killed male, survival is much shorter than if the same sperm is stored in vivo (Matei et al., 1980). In the experiment of Büyükhatoğlu and Holtz (1978) sperm survival was longer than in the present experiment: 9 days under air. Under O_2 fertility had dropped by nearly 20% in comparison with fresh sperm (controls). Other information was given in Stoss et al. (1978) showing that sperm survival lasted 23 days under O_2 but dropped after 15–17 days under air. Billard and Legendre (1980) observed that decline of fertilizing ability at $10^\circ C$ was more rapid when sperm was left in open vials (2 h) than when vials were filled with O_2 (6 h). Moreover, dissolved O_2 dropped after 4 h in an open vial and remained stable in vials filled with O_2 .

Other information shows that sperm survival in salmonids is very short in vials filled to the top (Smith and Quistorff, 1943; Barrett, 1951; Henderson and Dewar, 1959; Withler and Humphreys, 1967) or under N_2 or O_2 atmospheres (Büyükhatoğlu and Holtz, 1978; Stoss et al., 1978). Taking all this

together indicates that O_2 is involved in sperm survival and, as a result of respiration, O_2 drops in the medium and survival is limited in time. It should be pointed out that sperm concentration is very high, $10\text{--}25 \times 10^9$ spermatozoa/ml (Ginsburg, 1968; Billard et al., 1971), leading to a rapid exhaustion of O_2 in the medium.

An alternative way would be to dilute sperm prior to storage in a diluent which does not initiate sperm motility. Such diluent may include K^+ (Billard and Jalabert, 1974) or sucrose (Billard, 1980). This was tried by Büyükhatipoglu and Holtz (1978) without success but they used ordinary Cortland medium for dilution. Truscott et al. (1968), Sanchez-Rodriguez and Billard, 1977) also diluted salmonid sperm to include cryoprotector for storage at sub-zero temperature.

The variability of fertilizing capacity observed when pooled sperm is used to fertilize different females requires attention. It shows a type of incompatibility of gametes which is enhanced with time of preservation and which is not overcome, at least at the beginning, by an increase in the number of spermatozoa used for insemination. There was no way to estimate the quality of eggs prior to fertilization, although overmature looking females were discarded. It may correspond to a difference in the age of the eggs; ovulation occurred any time during the week preceding the start of the experiment and possibly the older eggs from females that ovulated early during this week were of poor quality. The difference was not very big at the beginning but was rapidly amplified. Eggs also aged during the experimental period, but this was not noticeable when they were fertilized with freshly collected sperm (see controls in Fig. 2). Heterogeneity in the quality of eggs right from the beginning of the experiment is possible; the females used in this experiment were young (first reproductive cycle) and Escaffre et al. (1977) have shown that survival of eggs in the body cavity is not as good in young as in older females. The plastic bags used for storing sperm in the present experiment are quite handy, they are easy to fill with O_2 and there is no risk of excessive evaporation. They may be used for sperm transportation for instance. But the practical application of such a technique is still limited because of the problems encountered in using the stored sperm with eggs of variable quality.

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