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ORIGINAL ARTICLE

Shelf life of embryo culture media: Buffering potential of media apparently not the determining factor

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KEYWORDS

Culture media;
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Buffering capacity;
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Abstract *Objective:* To investigate if the buffering power of embryo culture media is compromised by expiration.
Design: Buffering potential of expired culture media was evaluated by measuring pH values.
Setting: Fertility Laboratory, the University of Texas Medical Branch, USA.
Materials and methods: The pH of expired culture media were measured and compared with those of unexpired ones in three experimental conditions: (1) pre-incubation, (2) CO_2 incubation and (3) post-incubation. Sequential media, comprising Quinn's advantage fertilization, cleavage and blastocyst media (Sage assisted reproduction products Inc.), were used. The media were divided into expired and unexpired groups, based on the manufacturer's indicated expiration dates. The unexpired group was evaluated in their original condition (first time opened). The expired media bottles underwent more than one opening. The pH of the media was measured sequentially in conditions 1, 2 and 3.
Results: The expired media (30–390 days) displayed significantly ($p < 0.05$) different pH values compared to unexpired media in pre-incubation condition. However, expired media were capable of yielding similar pH values ($p \geqslant 0.23$) to those of the corresponding unexpired controls in CO_2

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incubation as well as post-incubation suggesting that the buffering capacity of the media is apparently not affected by expiration.

Conclusion: Time-dependent nutrient depletion, not the weakening of the buffering system, is probably the determining factor in setting the shelf life of the culture media.

1. Introduction

The buffering capacity of the culture media, used in assisted reproductive procedures, is critically important. It assures and maintains the required pH in the culture system. The biochemical mechanisms involved in the buffering of the culture media are well documented (1–4). The bicarbonate buffer emerged as the ideal buffering agent for the mammalian cell culture including human embryo culture (5–7). If media under incubator conditions fail to provide the expected pH due to its suboptimal buffering capacity, optimum embryo growth cannot be expected (8–11). Efficient buffering potential of the media secures a stress-free environment for optimum embryo development so that the desired end result of procedure, implantation and pregnancy is achieved (3,12,13). Realizing the importance of pH in embryo culture media, the laboratory personnel monitor the pH in the culture system, either directly or indirectly (3,12,14). In direct monitoring, the pH of the media is measured by a pH meter (12–14). In indirect monitoring, the CO_2 level in the incubator is measured to predict the pH of the media (4,7,12).

The required level of CO_2 is empirically determined in each laboratory setup, taking into consideration that the CO_2 concentration that yields a specific pH value can vary geographically with the altitude of the region where the laboratory is located (3,7). Furthermore, different makes of media may require different concentrations of CO_2 to attain the desired pH at a given altitude (12,14). The bottom line is that no matter what method is followed for pH monitoring, maintaining an optimum pH in the culture system is absolutely critical.

Fertility laboratories all over the world rely heavily on the utilization of commercially available media for assisted reproductive procedures (12,15). Several media manufacturing companies in the United States and in Scandinavian countries such as Sweden and Denmark have worldwide distribution centers (15). Currently, a vast majority of fertility clinics across the globe utilize the media produced by these international media companies (7,12,15). A common characteristic of all these commercial media is that they possess a short life span, usually expiring in 3–4 weeks. From a regulatory point of view it is illegal to use expired media in clinical procedures involving patients (16,17). Thus, by setting such a short shelf life, the media manufacturers are indirectly forcing the media users to discard a considerable amount of the unused media. Two vital issues, depletion of nutrients and weakening of the buffering capacity as time progresses, probably guide the manufacturers to set the shelf life for the safe use of the media. In this study, we investigated whether or not the buffering capacity of commercial media is compromised by the expiration dates indicated by the manufacturers.

2. Materials and methods

The pH of expired culture media were measured and compared with those of unexpired ones in three different experimental conditions to get an answer to the question raised above. Sequential media, comprising Quinn's advantage fertilization (Q-fert), Quinn's advantage cleavage (Q-cleve) and Quinn's advantage blastocyst (Q-blast) obtained from SAGE assisted reproduction products Inc. (Cooper Surgical Company, Trumbull, CT), were used in the study. The media were divided into expired and unexpired groups, based on the manufacturer's indicated expiration dates. The media bottles were stored in the refrigerator at 4 °C keeping the caps properly tightened during use and also after expiration. Media type, lot number, expiration date, and the volume remaining in the bottles were documented prior to pH measurement. The unexpired group was evaluated in their original condition (first time opened). The expired media bottles underwent more than one opening. Along with the removal of a variable amount of media for use, the rest were stored before they were brought under investigation. In both cases, the media bottles were opened as needed but for only short periods of time ($\leqslant$15 s in each incident).

The pH of the media was measured under the following three conditions. Condition 1: media bottles with caps tightened were kept out of the refrigerator until the media reached ambient temperature (23–24 °C). Condition 2: media volume of 5 mL in Falcon tubes was kept in the incubator (37 °C and 6% CO_2) with caps loosened for 26 h. Condition 3: incubated media (condition 2) were stored in the refrigerator with caps tightened for 72 h and then brought back to ambient temperature. The pH measurement was performed sequentially in conditions 1, 2 and 3, respectively, following the manufacturer's instructions to use the Orion 410+ pH meter (Thermo electronic corporation). A total of 10 unexpired and 85 expired media bottles were analyzed in condition 1. For conditions 2 and 3, 10 media bottles in each media type (Q-fert, Q-cleve and Q-blast) reflecting $\geqslant$30 days of post-expiration were selected from 85 expired media for comparison with the unexpired controls. Data were expressed as the mean ± SD. Analysis of variance, which included *t*-test and correlation coefficient, was used for statistical analysis with Sigma Plot software, version 11.0 for Windows. $p < 0.05$ was considered statistically significant.

3. Results

The pH values recorded for unexpired and expired media are shown in Table 1. The expired media, in condition 1, exhibited significantly ($p \leqslant 0.05$) higher pH compared to that of unexpired ones. The Q-fert, Q-cleve and Q-blast revealed pH of 7.77 ± 0.15, 7.75 ± 0.15 and 7.72 ± 0.12 in the expired state, and 7.29 ± 0.03, 7.23 ± 0.04 and 7.27 ± 0.04 in the unexpired