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Impact of a deep saturation dive on semen quality

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Summary

The demonstration dive 'Aurora' has provided an opportunity to study the impact of extreme hyperbaric conditions on male fertility. This operation involved a 33-day diving programme during which divers were exposed to a maximum pressure of 4.6 Mega Pascals (Mpa) for 7 days. At days – 4, + 27, + 34, + 82 and + 263 relative to the initiation of the dive, semen samples were analysed to determine the quality of spermatogenesis and the functional competence of the spermatozoa. A dramatic fall in semen quality was observed in association with the dive and by day + 82 the potential fertility of the men was seriously compromised as evidenced by oligoasthenoteratozoospermic semen profiles and the poor fertilizing potential of the spermatozoa. These studies indicate, for the first time, that the severe hyperbaric conditions associated with deep saturation dives have a profound effect on male reproductive function.

Keywords: hyperbaric conditions, male fertility, saturation dive, sperm function

Introduction

Further exploitation of the world's offshore oil and gas deposits depends upon the development of new technologies to support the exploration and recovery of hydrocarbons at deep water sites in excess of 400 metres of sea water (msw). An important element of this initiative has been to evaluate the safety and performance of divers working at the limits of human endurance, where the pressures may exceed 4 Mega Pascals (MPa) and the blood gases become saturated with oxyhelium (deep saturation dives). The pathological consequences of exposing divers to such extreme conditions is of clear military and commercial significance. As a consequence of this interest, numerous investigations have dealt with the impact of saturation diving on various aspects of human cardiac, neural, and respiratory physiology (Madsen *et al.*, 1994). This report presents the first evidence that deep saturation dives may also have a profound effect on male reproductive health.

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Materials and methods

A unique opportunity to examine the influence of severe hyperbaric conditions on male reproductive health was afforded by the deep saturation dive 'Aurora' conducted by the National Hyperbaric Centre, Aberdeen and part funded by the EU Thermie programme. The diving chamber employed in this study consisted of a hydrostatic test chamber connected to a saturation system, comprising two six-man twinlock decompression chambers and a transfer chamber, which also provided diving bell access to the hydrostatic test chamber. Full details of the system are available at the national Hyperbaric Centre web site (http:// http://www.demon.co.uk/hyperbar/sat.htm). In this study, a group of four divers were exposed to a heliox breathing mixture and subjected to a compression programme during which they were exposed to pressures equivalent to 450 msw (4.6 MPa) for 7 days followed by two further 3-day storage periods at 308 and 200 msw, respectively,

(Fig. 1a). Allowing for decompression the total dive time was 33 days.

In order to evaluate the impact of such extreme conditions on male reproductive function, three of the four divers volunteered to produce semen samples immediately before, during and after the saturation dive. In addition, two of the divers produced samples 243 days after the beginning of the dive, in order to provide data on the long-term effects of saturation diving conditions on male fertility. A fifth diver provided specimens over the same duration of time but was not selected for the dive. All samples were transported to the laboratory in a storage medium recently developed and validated for the ambient temperature transportation of human semen (Allan *et al.*, 1997).

The samples were assessed to determine the volume of the ejaculate, the number of spermatozoa and the percentage of cells that were motile and exhibited a normal morphology (World Health Organization, 1992). According to guidelines laid down by the World Health Organization ejaculates were classified as 'oligozoospermic' when they possessed $< 20 \times 10^6$ spermatozoa//mL, 'teratozoospermic' when morphology declined to < 40% normal, and 'asthenozoospermic' when the percentage of motile spermatozoa was < 40%. The movement characteristics of the spermatozoa were assessed using a computer aided semen analysis (CASA) system (Hamilton Thorn motility analyzer, Version 7; Hamilton Thorn, Beverly, MA, USA) at a temperature of 37 °C using the following settings: minimum contrast, 12; minimum size, 3; low and high size gates, 0.4 and 1.6, respectively; low and high intensity gates, 0.5 and 2.0, respectively; non motile head size, 8 and non motile intensity, 201. The standard terminology for the criteria measured by CASA systems, as stipulated by the World Health Organization (1992) has been used and includes curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP) and straightness (VSL/VAP). 'Percentage rapid' represented cells moving with a VAP of > 25 μm/sec.

The functional spermatozoa were subsequently isolated from the ejaculate using a 2-step Percoll gradient comprising 44% and 88% Percoll (Pharmacia, Uppsala, Sweden). Isotonic Percoll was created by supplementing 20 mL of 10 × concentrated Earle's medium (Gibco, Paisley, UK) with 3 mL 20% Albuminar (Armour Pharmaceutical Company, Eastbourne, UK), a pasteurized human serum albumin preparation in an aqueous base stabilized with 16 mM sodium caprylate and 16 mM sodium acetyltryptophanate, 6 mg sodium pyruvate, 0.74 mL of a 60% sodium lactate syrup, 2 mL of penicillin/streptomycin (10 000 IU/mL penicillin and 1000 μg/mL streptomycin; Gibco) and adding 180 mL Percoll (Pharmacia, Uppsala, Sweden). The medium used to dilute the isotonic Percoll was Biggers Whitten and Whittingham medium (BWW) supplemented with 20 mM HEPES and 0.3% Albuminar (Biggers *et al.*, 1971). Semen

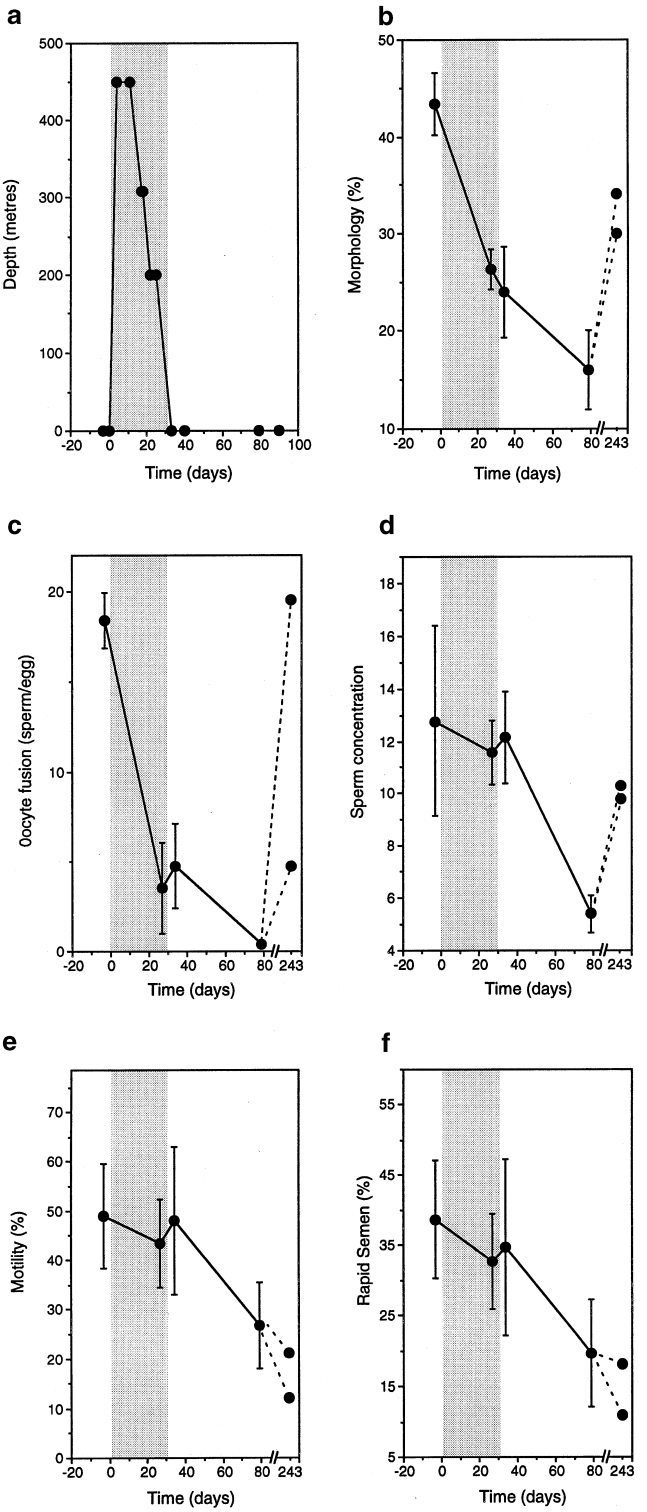

Figure 1. Influence of deep saturation diving on male reproductive health. (a) profile of the saturation dive; (b) sperm morphology; (c) sperm-oocyte fusion following activation with A23187; (d) sperm concentration; (e) percentage motile; (f) percentage rapid spermatozoa exhibiting average path velocities of > 25 μm/sec. The shaded area corresponds to the duration of the dive. Data points refer to mean ± SEM for three samples; dotted lines indicate the long-term changes observed in two divers who were available for a follow-up analysis.

Table 1. Variation in semen quality in relation to a deep saturation dive*

Variable	Day – 4	Day 27	Day 34	Day 82
VAP (μm/sec)	45.0 ± 1.0	39.4 ± 3.2	35.3 ± 3.84	43.3 ± 2.1
VSL (μm/sec)	35.7 ± 2.6	29.3 ± 2.9	27.3 ± 3.8	31.3 ± 4.9
VCL (μm/sec)	53.7 ± 1.0	49.9 ± 3.3	47.1 ± 5.0	48.2 ± 2.3
STR (%)	78.7 ± 4.6	72.0 ± 1.1	74.0 ± 0.1	73.4 ± 6.9
Zona binding (% Control)	89.2 ± 7.9	88.7 ± 12.4	71.0 ± 9.6	58.6 ± 4.2
ROS basal (Log cpm)	5.1 ± 0.1	4.9 ± 0.1	4.9 ± 0.2	5.3 ± 0.1
ROS FMLP (Log cpm)	5.8 ± 0.2	5.5 ± 0.3	5.5 ± 0.4	5.8 ± 0.1
ROS PMA (Log cpm)	6.7 ± 0.2	6.5 ± 0.4	6.2 ± 0.4	6.7 ± 0.3

*Dive covered days 0–33. Values represent mean ± SEM; $n = 3$.
FMLP, formyl –methionyl-leucyl-phenylalanine; PMA, 12-myristate; 13, acetate phorbol ester.

(1–3 mL) was layered on the top of each gradient and centrifuged at 500 *g* for 20 min. Purified populations of highly motile spermatozoa were subsequently recovered from the base of the 88% Percoll fraction, washed with 7 mL BWW, centrifuged at 500 *g* for 5 min and resuspended at a concentration of 2×10^7/mL.

The functional competence of these cells was then assessed by evaluating their ability to bind to salt stored human zonae pellucidae after 3 h incubation and to fuse with the vitelline membrane of the oocyte. For the latter, a heterologous in vitro fertilization system was used in which the ionophore A23187 was used to induce the acrosome reaction, as described by the World Health Organization (1992). The isolated spermatozoa were diluted 1:1 with the ionophore A23187, formulated as the free acid and prepared as 100 mM stock solution in dimethyl sulphoxide and diluted to give a final concentration of 2.5 μM. The spermatozoa were incubated with the ionophore for 3 h at 37 °C in an atmosphere of 5% CO_2 in air and were then pelleted by centrifugation at 500 *g*, resuspended in the same volume of fresh BWW and distributed as 50 μL droplets under liquid paraffin. Zona-free hamster oocytes were prepared as described (Yanagimachi *et al.*, 1976; Aitken *et al.*, 1993) and dispensed into the droplets at five oocytes per drop and 15–20 oocytes per sample. After a further 3 h, the oocytes were recovered from the droplets, washed free of loosely adherent spermatozoa, compressed to a depth of about 30 μm under a 22 × 22 mm coverslip on a glass slide and assessed for the presence decondensing sperm heads, with an attached or closely associated tail, by phase contrast microscopy. The number of spermatozoa penetrating each oocyte was assessed and the results expressed as the mean number of spermatozoa penetrating each oocyte (total number of penetrations per total number of oocytes).

Reactive oxygen species generation by the Percoll-purified sperm populations was assessed using a chemiluminescence technique employing the combination of luminol and horse radish peroxidase described by Aitken *et al.* (1992). The agonists used were 12-myristate, 13-acetate phorbol ester (PMA) to monitor the total free radical-generating capacity of the sperm suspension and formyl methionyl leucyl phenylalanine (FMLP) to assess the contribution due to infiltrating polymorphonuclear leukocytes (Krausz *et al.*, 1992).

Results

A summary of the findings of this investigation is given in Fig. 1. A dramatic fall in the quality of sperm morphology (Fig. 1b) was evident as early as 27 days after the initiation of the dive and continued to decline dramatically thereafter, generating a clear teratozoospermic profile by day + 82. During the course of the dive, these morphological changes were mirrored by a rapid and substantial decline in the capacity of acrosome reacted spermatozoa to fuse with the vitelline membrane of the oocyte (Fig. 1c). This criterion of sperm function also continued to decline after the end of the dive such that by day + 82 the spermatozoa had lost virtually all capacity for fertilization (Fig. 1c). At this point the spermatozoa had also suffered a significant decline in their capacity for binding to the zona pellucida which had declined to 58% of control levels (Table 1). Sperm concentration did not change for the duration of the dive but by day + 82 it had declined to oligozoospermic levels (Fig. 1d). Sperm motility exhibited a similar pattern of response, being unchanged for the duration of the dive but then declining to asthenozoospermic levels between days 34 and 82 (Fig. 1e). The quality of sperm movement also declined over the same time course in terms of the percentage of cells exhibiting rapid movement (VAP > 25 μm/sec; Fig. 1f,g). However, the mean values for the continuous velocity measurements (VAP, VCL, VSL) remained unaffected (Table 1). Although the functional competence of human spermatozoa is frequently compromised by oxidative stress, we could not detect an increase in free radical generation by these sperm suspensions at any point during or after the dive (Table 1).

As a consequence of all these changes, by day + 82, 48 days after the termination of the dive (Fig. 1a), the impaired functional competence of the spermatozoa and the oligoasthenoteratozoospermic semen profiles were indicative of severe infertility in all three participants in the saturation dive. In only one of the two divers assessed at day + 243, had the fertilizing potential of the spermatozoa returned to normal and in both cases sperm morphology and motility remained pathologically poor (Fig. 1b,e,f) even though sperm concentration had returned to the normal range (Fig. 1d). Intriguingly, the diver who was not selected for the dive exhibited oligoteratozoospermia (sperm concentration $< 20 \times 10^6$/mL, morphology < 40% normal, motility > 40%) at the beginning of the study and consistently maintained this profile throughout the 9-month period of observation.

Discussion

The Aurora project has provided a unique opportunity to examine the effects of deep saturation diving on potential male fertility. The preliminary results presented in this report indicate a profound, and possibly long-term, influence of deep saturation diving on male reproductive health. This is the first report to indicate a possible negative impact of severe hyperbarism on reproductive function in man. Although the number of subjects examined in this study may seem limited, the logistical difficulties and expense of launching a project such as Aurora should be recognized. This project cost £1 million to instigate and involved the co-operation of 28 separate companies as well as the EU. Moreover, the effects observed were not subtle. In all the divers examined the disruption of semen quality was clearly evident and was reflected in a dramatic loss of sperm function in association with the dive. Extensive research has been carried out into the impact of saturation diving conditions on other aspects of human physiology, particularly impaired neurological function, however, the fertility status of divers exposed to such conditions has not previously been addressed. At the very least these results alert us to the need to conduct further studies on the impact of saturation diving conditions on male fertility so that the occupational risk of such operations can be fully characterized.

What little data there are on the relationship between hyperbarism and testicular function were obtained in experiments conducted many years ago on rodents. These studies clearly indicated that exposure to heliox at high pressures could impair male fertility although the causative mechanisms were never resolved (Baden *et al.*, 1981; Hawley *et al.*, 1986; Fraser *et al.*, 1986). Short durations of exposure sufficient to affect only epididymal spermatozoa were without effect, suggesting that the subfertility observed under these conditions involved a subtle disruption of spermatogenesis (Fraser *et al.*, 1986). This conclusion is supported by the results obtained in the present study since the most dramatic changes in sperm number and motility were observed 40–80 days after the initiation of the dive (Fig. 1), commensurate with an effect at an early stage in spermatogenesis. In man, 64 days are required for the completion of spermatogenesis followed by approximately 10 days in the epididymis to induce maturation of the spermatozoa prior to ejaculation. Thus, if the week-long exposure to 4.6 MPa had affected the earliest stages of spermatogenesis, the consequences would not be apparent in the ejaculate for around 74 days, as was observed.

In addition to these affects on spermatogenesis, shorter-term effects targeting the late stages of spermiogenesis (the differentiation of a spermatozoon from a round spermatid) were also evident, judging from the abnormalities of sperm morphology observed just 27 days after the initiation of the dive. The dramatic fall in the fertilizing potential of the spermatozoa in the sperm-oocyte fusion assay (Fig. 1c) may also reflect an impact of hyperbarism on spermiogenesis or, possibly, on the free gamete following spermiation. This disruption of the fertilizing potential of human spermatozoa following a hyperbaric insult is in keeping with the results obtained by Fraser *et al.* (1986) who observed that spermatozoa recovered from mice exposed to high pressures exhibited a diminished capacity for fertilization associated with a failure of capacitation. This inability to capacitate was characterized by a reduced ability to exhibit both hyperactivated motility and the acrosome reaction (Fraser *et al.*, 1986).

Although animal studies have suggested that the subfertility induced by exposure to hyperbaric conditions is reversible (Dore *et al.*, 1983), it is of interest that the spermatozoa from one of the divers had still not recovered their capacity for fertilization, several months after the termination of the dive (Fig. 1c). Furthermore, the two divers subjected to long-term follow-up exhibited a sustained loss of sperm motility (Fig. 1e,f) and subnormal sperm morphology (Fig. 1b). It may also be significant that the diver who did not participate in the saturation dive was found to exhibit oligoteratozoospermia on entry into the study. These observations raise fundamental questions about the safety of saturation dives and, given that most divers are young men of reproductive age, emphasize the need for continued research into the health risks associated with occupational exposure to severe hyperbaric conditions.

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