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1 **Sperm cryostorage in a dry tank: An accurate alternative**

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25

26 **Highlights:**

27 Post-thawing sperm parameters, acrosome reacted and DNA fragmentation sperm are similar
28 after storage in dry or liquid nitrogen tank for normozoospermia and altered sperm samples.

29 Dry nitrogen tank conserves a stable temperature at -194°C despite repeated opening whatever
30 the storage floor level.

31 Sperm storage in dry tank is safety for samples and operators.

32

33 **Abstract**

34 This prospective study aimed to determine the effects of dry nitrogen cryostorage on human
35 sperm characteristics in comparison with liquid nitrogen cryostorage. For this purpose, 42 men
36 undergoing routine semen analysis (21 normozoospermia and 21 with altered semen
37 parameters) were analyzed. After slow freezing, half of the straws of each sample were
38 randomly stored in liquid and dry tanks, at the top and bottom levels of the latter. After 6
39 months storage, thawed samples were treated by density gradient centrifugation and sperm
40 characteristics were compared. There was no difference in sperm progressive motility
41 ($15.1\% \pm 14.2\%$ vs. $15.1\% \pm 12.7\%$; $p=0.76$), sperm vitality ($25.5\% \pm 17.7\%$ vs. $26.2\% \pm 19\%$;
42 $p=0.71$), percentages of acrosome-reacted spermatozoa ($38\% \pm 8.5\%$ vs. $38.5\% \pm 7.4\%$;
43 $p=0.53$) and DNA fragmentation spermatozoa ($27.3\% \pm 12.4\%$ vs. $28.5\% \pm 12.9\%$, $p=0.47$)
44 after cryostorage in the dry or the liquid nitrogen tank. Moreover, we did not observe
45 differences between either cryostorage system for normal and altered sperm samples. This
46 lack of difference was also observed whatever the floor level of cryostorage in the dry tank.
47 The temperature measurement of the dry tank showed a stable temperature at -194°C
48 throughout storage whatever the storage floor level, guaranteeing the stability of the low
49 temperatures suitable for human sperm storage. Because of its greater safety, dry storage
50 without contact with the liquid phase should be preferred and can be a useful alternative for
51 the cryostorage of human sperm samples.

52

53 **Key words:** cryostorage, human sperm, dry tank, liquid nitrogen tank, motility, vitality,
54 acrosome integrity, DNA fragmentation.

55

56 **1. Introduction**

57 Sperm cryopreservation is widely used for fertility preservation and sperm donation. Usually,
58 the storage method used is liquid nitrogen to avoid temperature variation and guarantee a
59 constant low storage temperature (- 196° C) necessary to preserve sperm motility [16,27],
60 DNA integrity [10,11] and fertilizing ability [1,12]. However, it has been reported that
61 infectious pathogens such as hepatitis B virus, bacteria and different fungi cannot only survive
62 but can also cross-contaminate cryopreserved samples in liquid nitrogen [5,14]. Therefore,
63 storage in systems that do not require the immersion of the materials in liquid nitrogen was
64 developed. Previous studies reported cryostorage systems in the vapor phase of nitrogen
65 [2,25]. Although storage in nitrogen vapor may represent an alternative for the cryostorage of
66 human sperm samples [17,22], this type of storage method is prone to the difficulties of
67 maintaining temperatures below -150°C between the high and low storage levels. Frequent
68 opening may also cause ice formation and frosting on lids and walls, and can thus attract
69 environmental microorganisms. To avoid these deleterious effects, cryostorage systems using
70 dry nitrogen tanks have been developed by manufacturers. Dry tanks permit storing samples
71 and liquid nitrogen in separate compartments. As the liquid nitrogen surrounds the dry
72 compartment containing the samples, the latter are maintained at a stable temperature close to
73 that obtained with a direct storage in liquid nitrogen. Nevertheless, the efficiency of this new
74 type of cryopreservation must to be evaluated for human sperm samples.

75 In the present study, to evaluate the efficiency of this new type of dry storage, we compared
76 the motility, vitality, acrosome integrity and DNA integrity of post-thaw sperm samples, after
77 sperm selection by density gradient that had been stored in dry and liquid nitrogen tanks. We

78 analyzed the stability of cryostorage after 6 months for the top and low level positions of the
79 samples in the dry tank.

80

81 **2. Material and methods**

82 **2.1 Experimental study design**

83 This prospective study was performed with 42 men (33.3 ± 4.4 years-old) undergoing routine
84 semen analysis for infertility at the center for reproductive medicine of the University Hospital
85 of Clermont-Ferrand, France. Written informed consent was obtained for inclusion of the
86 semen samples from these patients in the GERMETHEQUE biobank with the approval of the
87 local committee (Committee for Personal Protection DC 2008 558, Trial registration number:
88 NCT03734120). The flowchart of the experimental design is summarized in Figure 1. After
89 semen analysis according to the World Health Organization guidelines [28], the semen
90 samples were frozen by slow freezing. For each semen sample, the frozen straws were split in
91 two; one half was stored in the liquid nitrogen tank and second half in the dry tank. For the dry
92 tank storage, 18 of the 42 samples were stored at the top of the tank and 24 at the bottom. For
93 the both localization, we stored equal numbers of samples with altered and normal parameters.
94 The straws were thawed for evaluation of sperm motility, vitality, DNA integrity and
95 acrosomal integrity after 6 months storage.

96

97 **2.2. Semen collection**

98 Sperm samples were collected by masturbation into sterile plastic jars after 3-5 days of sexual
99 abstinence. After liquefaction at 37°C for 30min., they were analyzed according to the WHO
100 criteria [28] for routine semen analysis. The surplus semen was cryopreserved and analyzed
101 for this study.

102

103 **2.3. Sperm Cryopreservation methodology**

104 **2.3.1. Conventional slow freezing**

105 Each semen sample was mixed in a 1:1 ratio (v:v) with cryoprotectant medium (Cryosperm,
106 Origio®, France). The samples were maintained for 15 min at room temperature with
107 cryoprotectant medium and sealed in high security straws (Cryobiosystem®, L'Aigle, France).
108 Sperm freezing was performed, as described before (Brugnon *et al.*, 2013), using a
109 NanoDigitcool® programmable freezer (Cryobiosystem®, France).

110

111 **2.3.2. Sperm cryostorage**

112 After freezing, the straws, for each sample, were randomly stored in the dry tank (LO 2200
113 “dry”, Cryodiffusion®, France, Figure 2) and the liquid nitrogen tank (CF250M,
114 Cryodiffusion®, France) for 6 months. The straws were never in contact with liquid nitrogen
115 in the dry tank, since this type of tank is equipped with a separate compartment for liquid
116 nitrogen and another for sample storage. The specific external compartment of liquid nitrogen
117 surrounding the sample storage chamber allow maintaining a stable low temperature for
118 storage. As shown in Figure 2, the nitrogen level is managed by 4 temperature probes for both
119 types of tank with a high level detection probe (probe A), a stop filling detection probe (probe
120 B), a filling detection probe (probe C) and a low level detection probe (probe D). For both
121 tanks, automatic liquid nitrogen distribution occurs when temperatures higher than -196°C are
122 measured by probe C. When probe B detects -196°C, the automatic liquid nitrogen supply is
123 stopped automatically. In the dry storage unit, the 4 probes were localized in the specific
124 external compartment of the tank. Straws with normal and altered standard semen parameters
125 were stored both at the top and bottom of the dry tank. In the set of experiments, the real
126 temperature was measured in the storage compartment of the dry tank for 3 days with frequent
127 opening. The temperature measurements were performed with an external electric
128 thermometer (PT100 class A, precision of +/- 1.71°C for a temperature of -195.8°C,

129 Cryobiosystem®, France) at the same storage location of the sperm straws at the top and the
130 bottom of the tank (represented by stars in Figure2).

131

132 **2.3.3. Sperm thawing**

133 The straws were thawed at 37°C for 3 min. After the multi-step addition of an equal volume of
134 Sperm Preparation Medium (Origio®, France), the cryoprotectant was removed by density
135 gradient centrifugation with a two-step (95-47.5%) discontinuous Puresperm gradient
136 (Nidacon International®, Sweden) diluted in Sperm Preparation Medium. One ml of the
137 thawed sperm suspension was layered at the top of the gradient. After centrifugation (600g,
138 20min.), the purified sperm population was recovered from the 95% layer and washed in
139 Sperm Preparation Medium (750g, 8min.). The different sperm analyses were performed on
140 this sperm suspension.

141

142 **2.4. Analysis of sperm characteristics**

143 **2.4.1. Sperm motility and viability**

144 To evaluate sperm motility, 20µl of fresh and thawed semen was placed on the glass slide and
145 examined with phase contrast optics at x400 magnification. Two hundred spermatozoa were
146 assessed per duplicate to determine the percentage of progressive, non-progressive and
147 immotile spermatozoa [28]. The sperm vitality of fresh and thawed aliquots was estimated by
148 assessing sperm membrane integrity with eosin staining [28]. The sperm sample was mixed
149 (v/v) with eosin (Vital Screen, Fertipro®, Belgique) and examined by observation on glass
150 slides under a microscope at x400 magnification in duplicate to determine the percentage of
151 viable spermatozoa. Two hundred spermatozoa were assessed per duplicate.

152

153 **2.4.2. Sperm TUNEL assay**

154 As previously reported [7] DNA fragmentation in spermatozoa was detected by the TUNEL
155 assay (In Situ Cell Death Detection kit, Roche®, France). Briefly 1.5×10^6 washed sperm cells
156 were fixed with 2% paraformaldehyde for 30 min. at room temperature. Afterwards, the cells
157 were washed in PBS followed by permeabilization in 100µl of a solution containing 0.1%
158 Triton X 100 in 0.1% sodium citrate for 3 min on ice. Labeling was performed following a
159 washing procedure in PBS 1% BSA (500g, 5 min). Counterstaining with Propidium Iodide
160 (PI) allowed checking sperm permeabilization. For each sample, a negative control was
161 carried out by removing the TdT enzyme from the reaction mixture. The positive control was
162 obtained by incubating the spermatozoa with 2UI DNase for 15 min at 37°C in Tris HCl
163 buffer before labeling. Flow cytometry was performed using the flow cytometer LSRII (BD
164 Biosciences®, France). The sperm population was gated using the forward scatter signal and
165 the side scatter signal to exclude debris and aggregates. The excitation wavelength of 488nm
166 was supplied by an argon laser. Green fluorescence (FITC- derived fluorescence) and red
167 fluorescence (PI) were detected with FL1 (530/30nm) and FL3 (610/20nm) channels,
168 respectively. A minimum of 20 000 spermatozoa were examined for each assay.

169

170 **2.4.3. Sperm acrosome integrity**

171 Acrosome integrity was estimated as described previously [21]. Briefly, sperm cells were
172 fixed, permeabilized in methanol 98% (30min, -20°C) and labeled with a freshly prepared
173 solution of Pisum Sativum Agglutinin conjugated to fluorescein isothiocyanate (PSA-FITC,
174 Sigma-Aldrich®, France) (60µg /mL) in PBS. Sperm cells were observed blindly using
175 epifluorescence microscopy (x400). Sperm cells with intact acrosome membranes showed
176 marked fluorescence in the acrosome region (A pattern), while those having lost their
177 acrosome membrane were devoid of fluorescence or displayed marked fluorescence along the
178 equatorial segment (AR pattern). Two hundred spermatozoa were assessed per duplicate.

179

180 **2.5. Statistical analysis**

181 Sample size was determined according to Cohen's recommendations concerning effect-size (= 182 difference between two means divided by standard-deviation) bounds [9]. With 42 infertile 183 patients, an effect-size greater than a moderate value of 0.5 can be highlighted for paired 184 comparisons for a two-sided type I error at 5%, a power equal to 90% and an intra-individual 185 correlation coefficient at 0.5. With 24 and 18 patients for the top and bottom floor, 186 respectively, and 21 patients for each normozoospermia group, the expected effect-size was 187 around 0.8 for a power higher than 80%.

188 The statistical analysis was conducted using Stata software (version 13, StataCorp, US). The 189 tests were two-sided with a type I error set at 5%. Continuous data (progressive motility, 190 sperm vitality, percentages of acrosome-reacted sperm, sperm DNA fragmentation) were 191 presented as mean \pm standard-deviation. The comparisons between groups (top vs. bottom 192 floor, normal vs altered sperm parameters) were performed using the Student t-test or the 193 Mann-Whitney test. The paired comparisons (at 6 months of cryostorage, dry vs. liquid 194 nitrogen tank) were performed using the Student paired t-test or the Wilcoxon test. A Sidak 195 type I error correction was applied to take into account the multiple comparisons. Finally, 196 linear mixed models were carried out to evaluate the following effects – top/bottom, fresh / 197 dry / liquid, normal / altered sperm parameters, time and their interactions – taking into 198 account between and within subject variability as random-effect. When appropriate, a 199 logarithmic transformation was proposed to obtain the normality of the endpoint.

200

201 **3. Results**

202 **3.1. Sperm characteristics after cryostorage in dry and liquid nitrogen tanks**

203 The initial sperm parameters of the samples before freezing are presented in table 1. The 204 sperm concentration was $77.2 \pm 63.5 \times 10^6$ /mL, progressive motility $43.8 \pm 16.4\%$, sperm 205 vitality $76.5 \pm 13.4\%$ and the percentage of atypical sperm $8.9 \pm 6.4\%$. Normozoospermia was

206 observed in 21 samples and 21 samples showed altered semen parameters according to the
207 WHO criteria. Initial sperm parameters were not significantly different between samples
208 assigned to the bottom and top storage levels of the dry tank.

209 Figure 3 shows the characteristics of post-thaw human samples after 6 months' cryostorage in
210 dry and liquid nitrogen. After thawing, the sperm samples were treated by density migration
211 density as it is recommended to use sperm samples for assisted reproductive technology. Post-
212 thawed sperm progressive motility and vitality were significantly decreased for the both
213 storage types and whatever the initial sperm quality ($p < 0.001$, Figure 3A). There was no
214 difference in sperm progressive motility ($15.1 \pm 14.2\%$ for dry *vs.* $15.1 \pm 12.7\%$ for liquid
215 tank; $p=0.76$, Figure 3A) or sperm vitality ($25.5 \pm 17.7\%$ *vs.* $26.2 \pm 19\%$; $p = 0.71$, Figure
216 3A). Moreover, there was no difference between the percentages of acrosome-reacted
217 spermatozoa ($38 \pm 8.5\%$ *vs.* $38.5 \pm 7.4\%$; $p = 0.53$ Figure 3B) and DNA fragmentation
218 spermatozoa ($27.3 \pm 12.4\%$ *vs.* $28.5 \pm 12.9\%$, $p=0.47$, Figure 3C) in dry or liquid nitrogen.
219 There was also no statistical difference for any of the parameters measured between
220 cryostorage in dry or liquid nitrogen whatever the quality of the initial sperm
221 (normozoospermia and altered sperm samples represented by triangles and dots, respectively
222 in Figure 3).

223

224 **3.2. External temperature measurement of the dry tank**

225 The temperature of the storage compartment in the dry tank was measured using an external
226 probe for 3 days at the top and the bottom floor alternatively (the two positions are represented
227 by stars in Figure 2). During this period, the tank was opened frequently and filled daily. The
228 external probe showed stables temperatures of -194 -193 ° C for both positions
229 (supplementary figure).

230

231 **3.3. Comparison of sperm characteristics of samples stored at the top and low levels of**
232 **the dry tank with samples stored in the liquid nitrogen tank**

233 Figure 4 compares the characteristics of post-thaw human samples cryostorage during 6
234 months in the top and bottom floors of the dry vs. liquid nitrogen tank for the same sperm
235 samples. We did not observe any difference for sperm vitality ($25.6 \pm 17.8\%$ vs. $27.7 \pm$
236 19.4% , $p = 0.39$) nor progressive motility ($16.8 \pm 15.2\%$ vs. $16.2 \pm 13.2\%$, $p = 0.92$) between
237 the samples stored at the top floor of dry tank in comparison with liquid tank. Similarly, we
238 measured any difference in bottom floor of the dry tank vs. liquid nitrogen tank for sperm
239 vitality ($25.4 \pm 18\%$ vs. $24.3 \pm 18.8\%$, $p = 0.67$) and progressive motility ($12.9 \pm 12.6\%$ vs.
240 $13.6 \pm 12.3\%$, $p = 0.68$). Moreover, there was no difference between the percentages of
241 acrosome-reacted sperm samples stored in the top floor of the dry tank ($36.1 \pm 7\%$ vs. $36.5 \pm$
242 6.1% for N_2 liquid storage, $p = 0.76$) and in the bottom floor ($40.4 \pm 9.7\%$ vs. $41.2 \pm 8.32\%$
243 N_2 liquid storage, $p = 0.55$) and in the percentage of spermatozoa with DNA fragmentation of
244 samples stored in the top floor ($30 \pm 13.5\%$ vs. $30.1 \pm 13.8\%$ N_2 liquid storage; $p = 0.94$) and
245 in the bottom floor ($26.4 \pm 12.5\%$ vs. $23.2 \pm 9.2\%$ N_2 liquid storage, $p = 0.28$) in comparison
246 with liquid nitrogen tank. This lack of difference was observed not only in normozoospermia
247 but also in altered sperm samples ($p > 0.05$).

248

249 **4. Discussion**

250 As an alternative to the standard method of human sperm storage in liquid nitrogen at a
251 temperature of -196°C , storage in a dry tank reduces the risks of cross-contamination and
252 avoids the risks incurred by manipulations of liquid nitrogen [20]. In our study, we
253 cryopreserved human sperm samples and stored them in dry and liquid nitrogen tanks for six
254 months, and evaluated the characteristics of post-thaw spermatozoa. We obtained similar
255 results for sperm motility, vitality, acrosome integrity and DNA fragmentation in both
256 cryostorage conditions. Moreover, there was no impact of the position of sperm samples in

257 either of the tanks on the sperm characteristics we measured, likewise for the
258 normozoospermia and altered sperm samples.

259 One of the disadvantages of using liquid nitrogen is that certain infectious agents not only may
260 survive but can also cross contaminate other samples stored in the same dewar unit during
261 long storage periods [6,26]. Indeed, contamination is possible if a contaminated sample is
262 stored in liquid nitrogen [13]. There was no report of cross-contamination in semen samples in
263 dry shippers [4]. Although the volume of the tank we evaluated in our study is larger, storage
264 in this type of tank should be done to ensure greater safety [6]. The separation wall between
265 the locations of the samples and the liquid nitrogen of the dry tank we tested guarantees the
266 absence of contact between liquid nitrogen and samples. To ensure security, different rules
267 should be applied along with the screening of patients for infectious diseases and the
268 application of the appropriate sanitary and cryoprocedures. Moreover, the periodic
269 disinfection of the tanks and the use of sterile liquid nitrogen is advisable [20].

270 In order to prevent all biological activity [19], the biological samples must be stored below the
271 glass transition temperature of water. Liquid nitrogen permits maintaining the temperature at -
272 196°C. The risk of thermal instability of the stored samples must be considered when using a
273 system with nitrogen in vapor phase. The dry tank we used in our experiment always
274 guaranteed a stable temperature of -194°C for the top and bottom floors of the tank despite
275 repeated openings. The location of the liquid nitrogen surrounding the location of the stored
276 samples permits high stability without nitrogen vapor in the sample storage compartment.
277 Moreover, the tank is connected for the detection of low-level filling and temperature
278 thresholds. With the development of vitrification cryoprocedures, particularly for oocyte and
279 embryo cryopreservation in reproductive medicine, it has been clearly demonstrated that it is
280 necessary to maintain vitrification by remaining at temperatures lower than -126°C [24].
281 Moreover, if a semi-open device is used for vitrification, using a dry tank avoids the risks of
282 cross-contamination.

283
284 Although cryopreserved semen is routinely used in assisted reproductive techniques,
285 freezing/thawing procedures induce alterations of sperm structures and functions. Sperm
286 motility is the most sensitive indicator of cryodamage. In our study, similar alterations of
287 sperm motility and vitality were measured after thawing sperm samples stored in dry and
288 liquid nitrogen tanks after 6 months storage whatever the level of storage in the tank. Several
289 groups reported similar results with vapor phase tanks [15,17,22]. The motile sperm recovery
290 rate after thawing may be improved by density gradient centrifugation performed before
291 freezing [7] and/or supplementation of the media by an antioxidant [3,18]. Indeed, sperm
292 treatment by density gradient centrifugation before freezing allows the removal of round cells
293 and the selection of mature and motile spermatozoa which seem more resistant to cryoinjury.
294 We performed density gradient centrifugation after thawing in our study because it is the
295 procedure applied most routinely in assisted reproductive techniques when using
296 frozen/thawed sperm samples.

297
298 In addition, it has been reported that sperm acrosome reaction [10] and DNA fragmentation
299 [8,29] could be modified by cryoprotocols. Indeed, cryopreservation procedures may induce
300 the phenomenon of capacitation and acrosome reaction. When this occurs, the spermatozoa
301 lose their fertility since the acrosome reaction is an irreversible phenomenon which normally
302 occurs during fertilization. In our study, the acrosome integrity of the thawed spermatozoa was
303 similar when storage was performed in both dry and liquid nitrogen tanks after 6 months'
304 storage, whatever the level of storage in the tanks (top or bottom). We performed TUNEL
305 assays to evaluate sperm DNA fragmentation. This technique permits the detection of double
306 strand DNA fragmentation which can be attributed to oxidative stress [29], apoptosis and/or
307 defective chromatin packaging [23]. We showed similar percentages of spermatozoa with
308 DNA fragmentation in semen samples stored in dry and liquid nitrogen with no impact of

309 storage duration or level in the tanks. Due to the large amount of spermatozoa necessary to
310 perform all these measurements, it was not possible to evaluate sperm acrosome integrity and
311 DNA fragmentation before freezing. The lack of difference for all the sperm characteristics
312 measured for the normozoospermia and altered sperm samples, guarantees efficient storage for
313 male fertility preservation and sperm donation in dry tanks.

314

315 **5. Conclusion**

316 The characteristics of the cryopreserved human semen samples in dry and liquid nitrogen
317 tanks were similar for sperm motility, vitality, acrosome integrity and DNA fragmentation
318 after 6 months' storage in the top and bottom floors of the tanks. Storage in a dry tank
319 guarantees the stability of the low temperatures suitable for human sperm storage. Because of
320 its greater safety, dry storage without contact with the liquid phase should be preferred and
321 can be a useful alternative for the storage of human sperm samples. In future, it could be
322 interesting to evaluate the impact of this type of storage on ART outcomes, particularly
323 fertilization, embryo development and pregnancy rates.

324

325

326 **Declaration of Competing Interest**

327 There was no external funding for this study and all the authors declare no competing interests

328

329

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336

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Table 1: Characteristics of non-frozen semen samples assigned to the top and bottom storage levels

	Total	Normozoospermia	Altered sperm parameters	Samples assigned to top storage level	Samples assigned to bottom storage level
n	42	21	21	18	24
Volume (mL)	4.2 ± 1.4	3.9 ± 1.1	4.4 ± 1.6	4.4 ± 1.3	3.8 ± 1.4
Concentration (10⁶/mL)	77.2 ± 63.5	95.6 ± 61.3	58.8 ± 61.7*	75.7 ± 72.5	79.2 ± 51.2
Progressive Motility (%)	43.8 ± 16.4	54.1 ± 9.3	33.6 ± 15.7*	39.4 ± 17.1	47.3 ± 15.3
Vitality (%)	76.5 ± 13.4	80.7 ± 8.6	72.2 ± 16	77.3 ± 10.7	75.3 ± 16.5
Teratozoospermia (%)	8.9 ± 6.4	11.6 ± 5.4	6.2 ± 6.3*	8.3 ± 6.2	9.8 ± 6.7

Data are presented as mean (\pm standard deviation). Paired comparisons were performed using the Student paired t-test and the Wilcoxon test (p-value). “Top floor stored samples” and “Bottom floor stored samples” columns correspond to sperm parameters before freezing of samples stored at the top and the bottom floor of the dry tank. * indicates significantly different values in comparison with normozoospermia sperm samples with a p-value < 0.05 .

Figure 1: Experimental design and flow chart of the study.

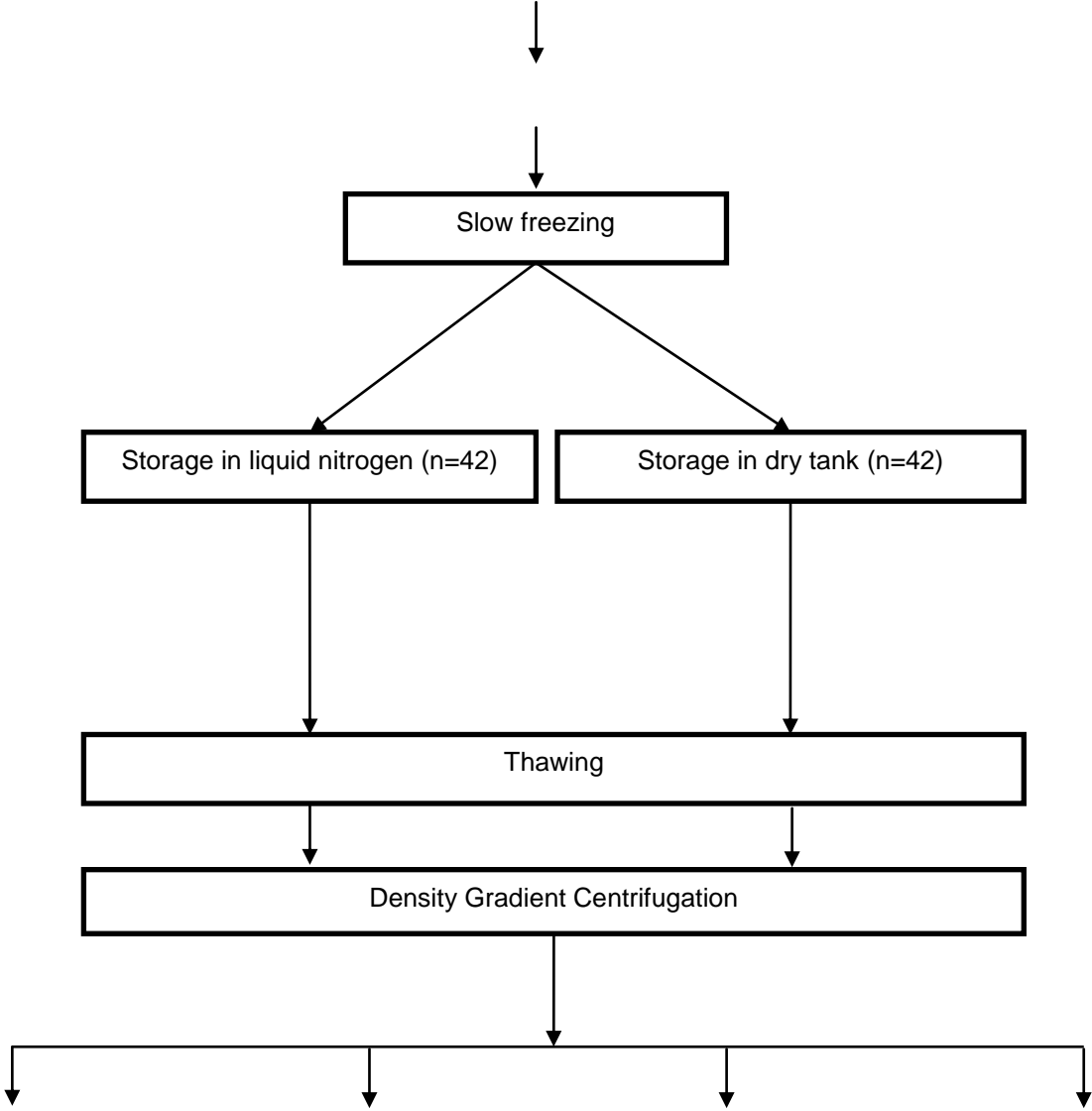


Figure 2: Characteristics of probes of the dry and liquid tanks.

The nitrogen level is managed by 4 temperature probes for dry (LO2200) and liquid (CF 250M) tanks: probe A: High level detection (61 cm from the bottom); probe B: Stop filling detection (56 cm from the bottom), probe C: Filling detection (48 cm from the bottom), D: Low level detection (45 cm from the bottom). In the dry storage unit, the 4 probes were localized in the specific external compartment of the tank.

Straws with normal and OAT semen were stored both at the top and bottom of the dry tank. In the set of experiments, the real temperature was measured in the storage compartment of the dry tank for 3 days with frequent opening. The temperature measurements were performed with an external electric thermometer (PT100 Cryobiosystem®, France) at the same storage location as that of the sperm straws at the top and the bottom of the tank (represented by stars).

	Dry tank	Liquid tank
Reference	LO2200 « Dry », Cryo Diffusion®,France	CF 250M, Cryo Diffusion®,France

Tank

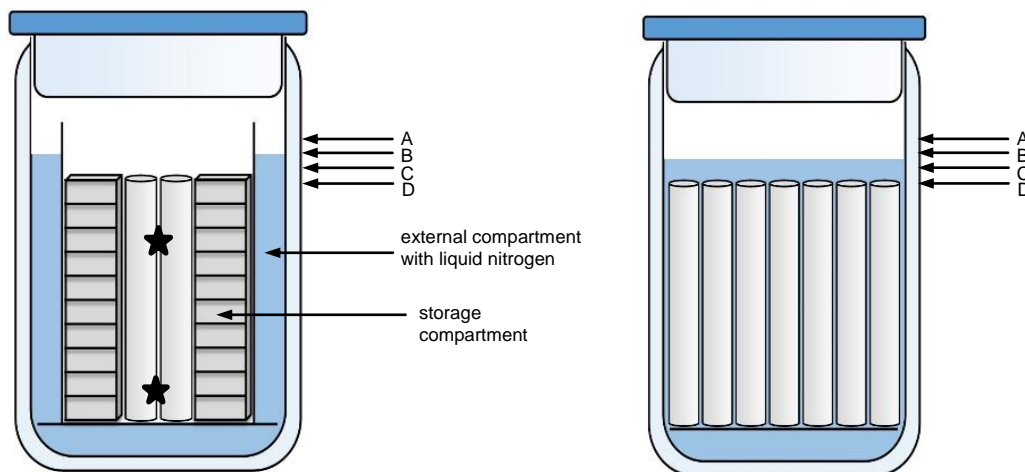
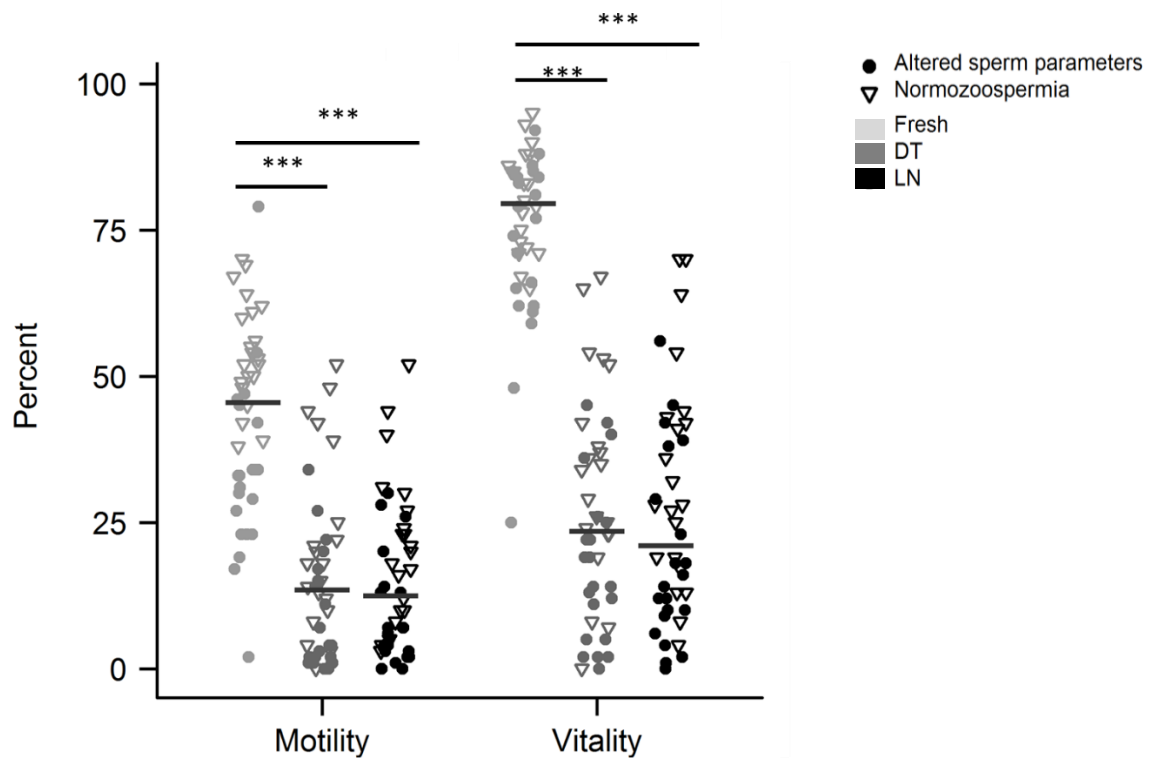


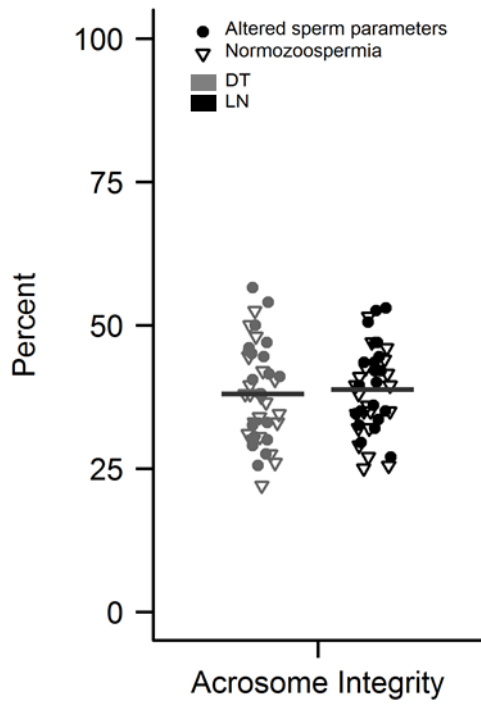
Figure 3: Post-thaw characteristics of sperm stored in a dry tank (DT) and liquid nitrogen (LN) after 6 months cryostorage.

Mean Percentages (line) of sperm progressive motility and vitality (in A, n=42 for both) acrosome integrity (in B, n=42) and DNA fragmentation (in C, n=22) are shown after 6 months' cryostorage in dry (dark gray dots and triangles) and liquid nitrogen (black dots and triangles). Parameters measured for normozoospermia and altered sperm patients are represented by triangles and dots, respectively. In A, sperm vitality and progressive motility were shown for fresh samples before freezing (light grey). Paired comparisons between DT and LN storage were performed using the Student paired t-test and the Wilcoxon test (p). *** indicates a significant difference with $p < 0.001$ in comparison with fresh samples.

A



B



C

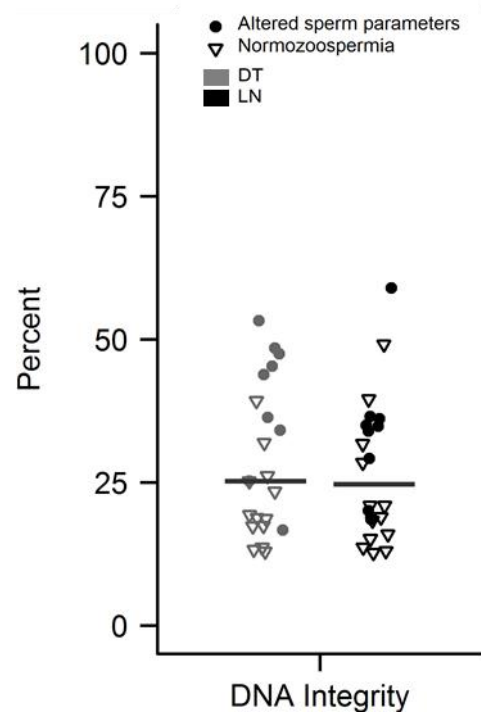
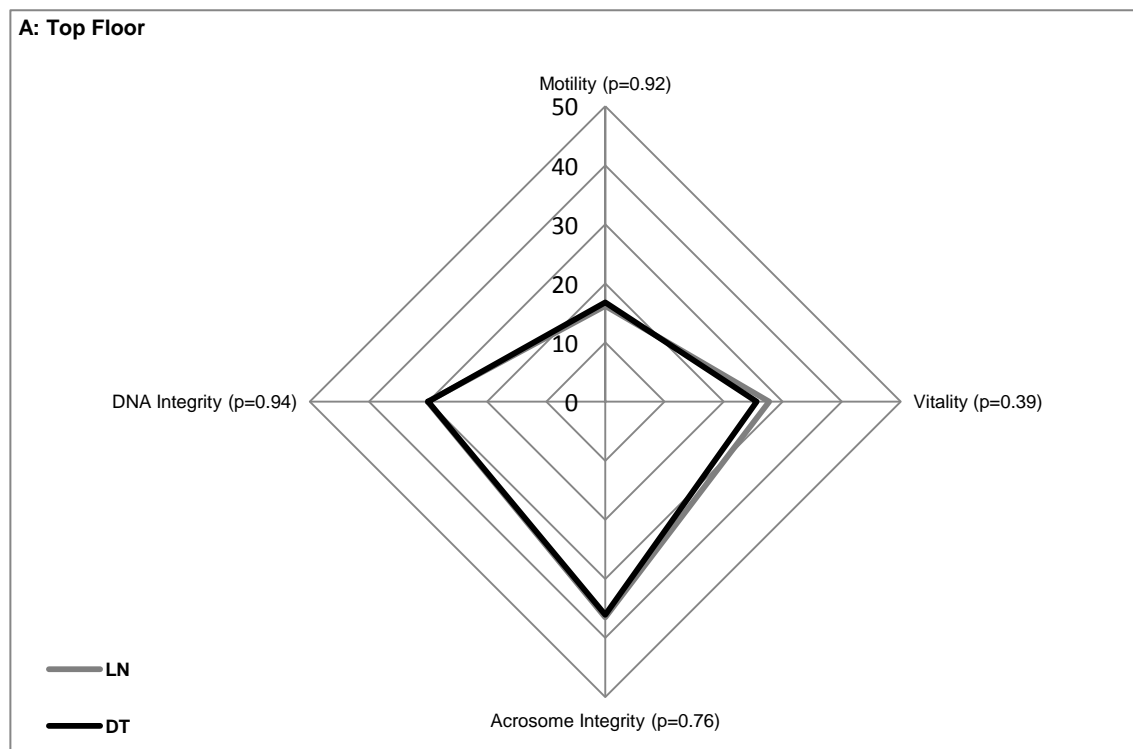


Figure 4: Post-thaw characteristics of sperm after cryostorage during 6 months on the top floor (A) and bottom floor (B) of the dry tank (DT) *versus* liquid nitrogen tank (LN)

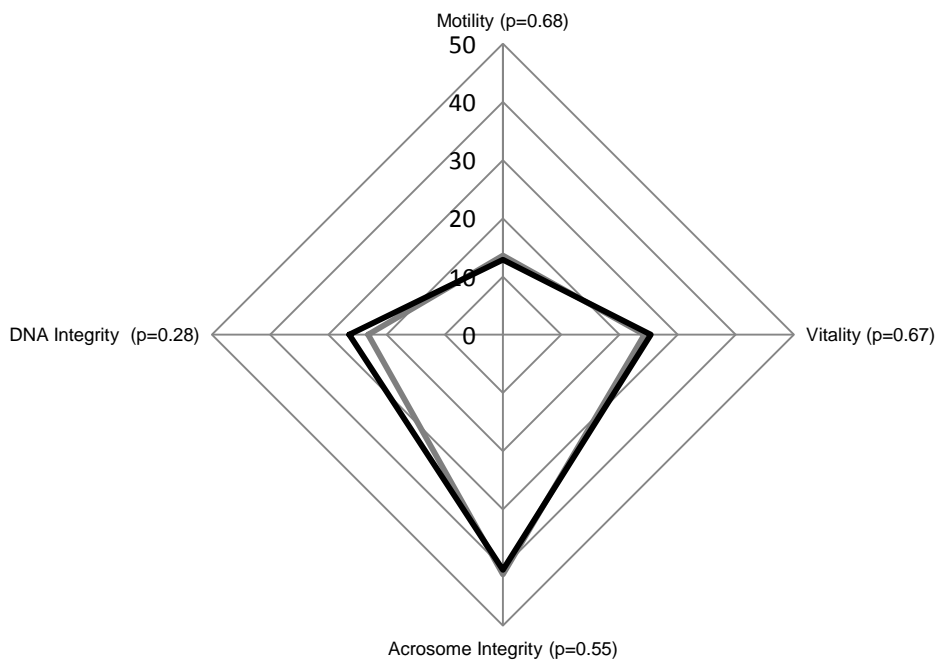
A: Average percentages of sperm vitality (n=24), progressive motility (n=24), acrosome integrity (n=24) and DNA fragmentation (n=13) of samples stored at the top (A) floor of the dry tank are shown in comparison with the liquid tank. The straws issue from the same sperm sample patient were compared in the two type of tanks (dry and liquid nitrogen).

B: Average percentages of sperm progressive motility (n=18), vitality (n=18), acrosome integrity (n=18) and DNA fragmentation (n=9) of samples stored at the bottom (B) floor of the dry tank are shown in comparison with the respective straws (from the same samples) stored in the liquid tank.

Paired comparisons were performed using the Student paired t-test and the Wilcoxon test (p).



B: Bottom Floor



— LN
— DT

Supplemental Figure: Temperature measurement of the dry tank

The temperature of the storage compartment in the dry tank was measured using an external probe (black line) for 3 days at the top and the bottom floor alternatively. During this period, the tank was opened frequently and filled daily. The temperature measured by the high level detection probe (probe A, grey line) and automatic liquid nitrogen distribution (grey arrow) were also represented.

