# Minitube TurboFreezer

## A standardized freezing process is crucial for efficient production of cryopreserved bull semen

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The objective of utilizing nitrogen vapour based programmable semen freezers is to obtain a controlled and standardized freezing process which maintains optimum quality of the semen. Unfortunately, most of these freezers work with only one liquid nitrogen inlet and fan. In fact, this is a design which produces an undefined vapor circulation with non-standardized speed and temperature throughout the chamber. This results in the formation of warm and cold spots depending on the location of straws in the freezing chamber with ever increasing temperature differences in large freezing chambers.

In contrast to these programmable freezers, the TurboFreezer exhibits a homogenous distribution of temperature in the freezing chamber (see in vitro study 1) due to its unique concept of directed horizontal air flow. Therefore, the TurboFreezer is the first real innovation in nitrogen based semen freezers since 30 years. The TurboFreezer in comparison to a conventional freezer has shown to lead to significantly superior post-thaw quality of bull semen.

### Field trial

On a commercial bull stud, semen was collected, processed, frozen, and analyzed after thawing. Ejaculates were collected once or twice per week from 26 bulls (primarily Simmental). Ejaculates meeting minimum standard requirements (total motility >70%) were processed (extender used: Steridyl®, Minitüb GmbH) and frozen by the laboratory`s standard procedure. For cryopreservation, two different freezing technologies were used in a split sample design. The effect of freezing technology on post-thaw semen quality (total motility, progressive motility and membrane integrity) was analyzed using a CASA system (AndroVision®, Minitüb GmbH).

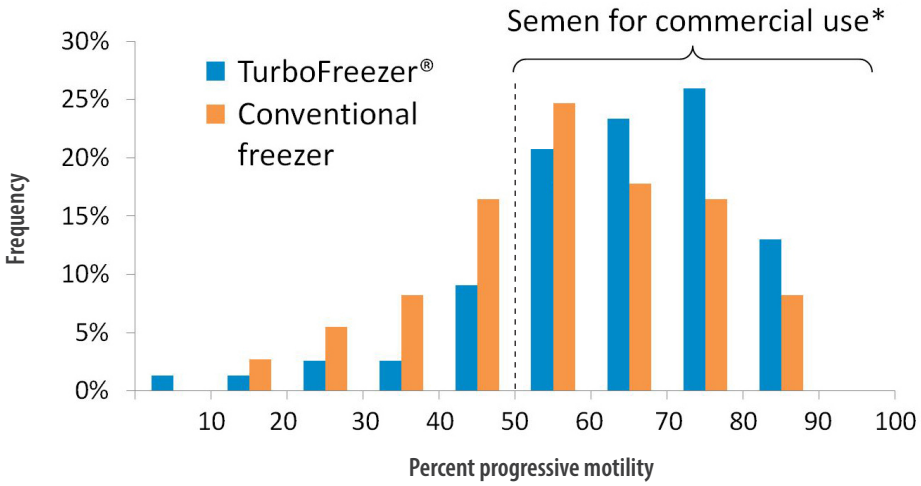
The results revealed a significant superiority regarding sperm motility parameters as well as membrane integrity ($p \leq 0.05$) in the TurboFreezer group of samples. In detail, post-thaw total motility was increased by 8.7%, progressive motility by 6.9% and sperm with intact membranes by 7.5% using the TurboFreezer versus the conventional freezer, respectively (see table 1).

| | TurboFreezer® | | | Conventional Freeezer | | |
|---|---|---|---|---|---|---|
| | x | sd | n | x | sd | n |
| Total motility (%) | 73.8[a] | ±15.7 | 233 | 65.2[b] | ±20.6 | 222 |
| Progressive motility (%) | 62.9[a] | ±17.7 | 233 | 57.0[b] | ±20.7 | 222 |
| Membrane integrity (%) | 79.3[a] | ±15.8 | 143 | 71.8[b] | ±17.8 | 187 |

(sd = standard deviation; a:b, $p<0.05$)

*Table 1: Post-thaw quality of semen samples according to freezing technique*

Furthermore, the number of ejaculates fulfilling minimum standards for progressive motility after thawing (≥50%) was 16% higher when frozen with the TurboFreezer. Consequently, efficiency is increased as fewer ejaculates are discarded after post-thaw quality control (see figure 1).




*According to recommendations of ADR (Arbeitsgemeinschaft Deutscher Rinderzüchter, Umbrella organisation of organized cattle production, Germany)

*Figure 1: Post-thaw progressive motility according to freezing technique*

**Technical background:**

Inside the TurboFreezer, four turbo injection valves with fans provide a powerful nitrogen vapor stream which is forced to flow horizontally through the racks holding the straws. The predefined speed of this vapor stream in combination with precisely controlled temperature leads to uniform and predefined cooling and freezing patterns of the complete batch of straws. Especially the instant removal of the crystallization energy results in an extremely short duration of the temperature plateau following crystallization which is unique and cannot be matched by any other available freezer until now.

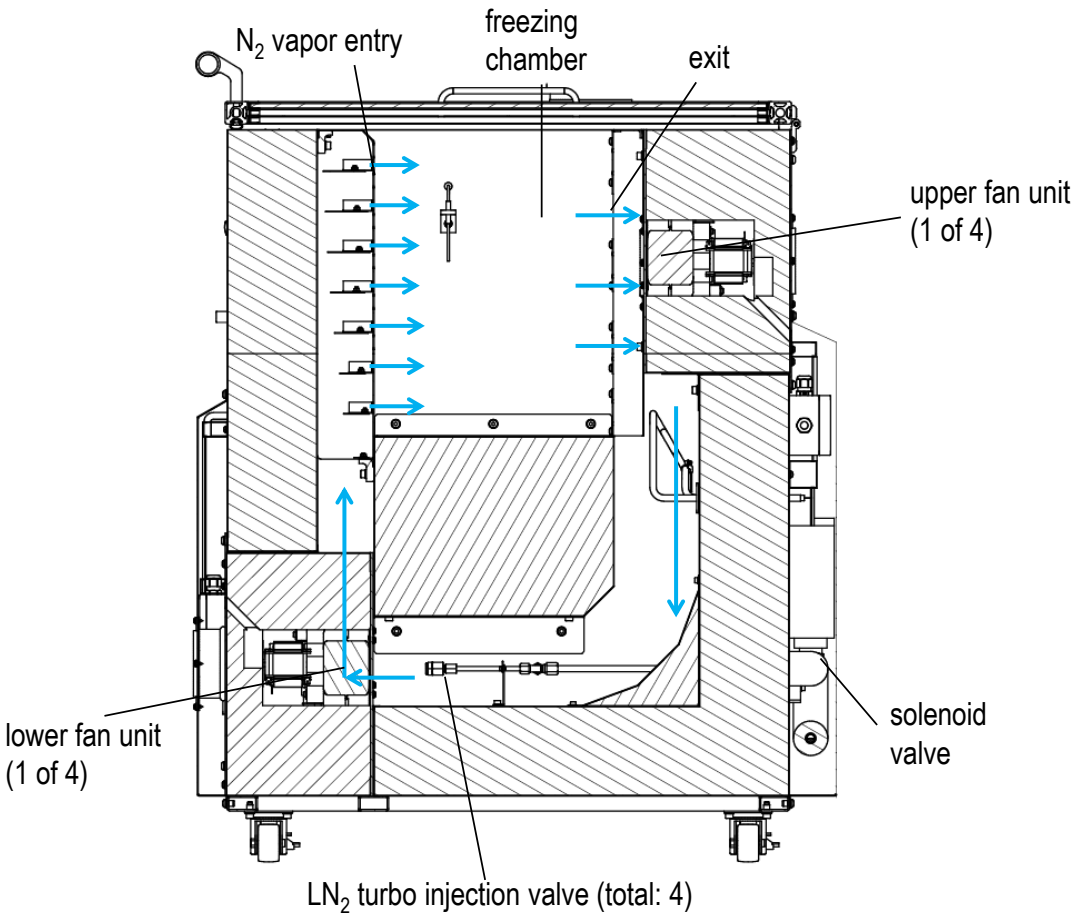



*Figure 2: TurboFreezer Vapor Circuit*

## Economic freezing performance in labour and $LN_2$ consumption

The TurboFreezer provides a dual operation lid (Figure 3): Inserted sliding covers for loading and unloading of racks avoid loss of cold vapor during handling. The second complete cover is supported by air pressure cylinders and allows an ergonomic and easy handling of racks and straws. There is ample space available to unload racks and to transfer straws into goblets inside the freezing chamber.

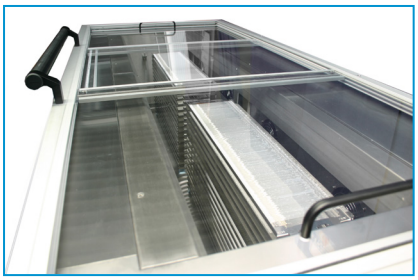



*Figure 3: Dual operation lid*

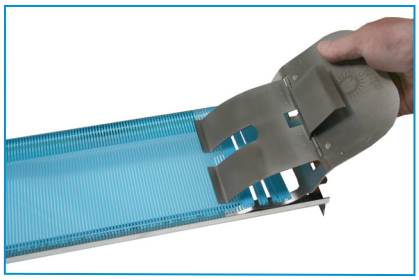


*Figure 4: Straw plier*

A newly developed straw plier (Figure 4) to unload frozen straws from the racks ensures optimal handling for the user whilst protecting the straws from accidental warming.

The Turbo freezing racks are streamlined for optimal vapor flow rate (Figure 5).
Low nitrogen consumption per freezing cycle combined with high through-put of straws per cycle provides an outstanding economic freezing performance in terms of labor and $LN_2$ consumption.

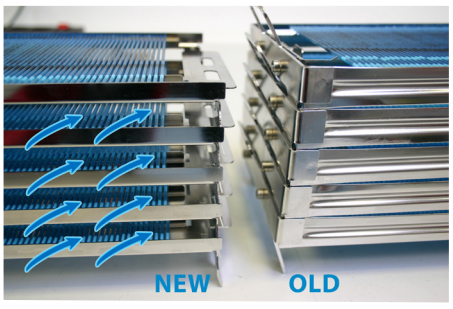



*Figure 5: Streamlined freezing racks*

## In vitro studies

### Study 1: Comparison of freezing cycles of different freezers

A crucial point for optimal freezing results of a semen batch is the precise control of the freezing pattern of each single straw by instant removal of crystallization energy. An important aim is therefore, to provide a temperature plateau duration which is as short as possible. To measure the temperature homogeneity at different points in the freezing chamber, test runs were performed. Freezers were filled with full racks according to their capacity. At different places on the racks, straws were equipped with temperature sensors. Factory default freezing curves were used.

Definition of terms:

- Cooling rate: Temperature decreases (°C per minute) to crystallization temperature.
- Plateau duration: Time until crystallization temperature is regained needed to come back to T Crystallization (B) after the temperature increase during plateau phase.
- T Crystallization (A): Temperature inside straw at the moment of crystallization.
- Freezing rate: °C per minute; the temperature inside the straws went down from the temperature at end of plateau until reaching -80 °C.

Results:

| Minitüb TurboFreezer: | Level 0 | Level 1 | Level 2 |
|---|---|---|---|
| Cooling rate [°C/min] | -34 | -36 | -42 |
| Plateau duration [s] | 59 | 53 | 40 |
| T Crystallization [°C] | -15 | -12 | -13 |
| Freezing rate [°C/min] | -74 | -74 | -65 |

| Conventional Freezer I: | Level 0 | Level 1 | Level 2 |
|---|---|---|---|
| Cooling rate [°C/min] | -44 | -8 | -13 |
| Plateau duration [s] | 15 | 51 | 61 |
| T Crystallization [°C] | -14 | -5 | -9 |
| Freezing rate [°C/min] | -159 | -53 | -14 |

| Conventional Freezer II | Level 0 | Level 1 | Level 2 |
|---|---|---|---|
| Cooling rate [°C/min] | -4 | -6 | -6 |
| Plateau duration [s] | 79 | 54 | 32 |
| T Crystallization [°C] | -15 | -11 | -13 |
| Freezing rate [°C/min] | -63 | -44 | -33 |

*Table 2: Comparison of temperature profile for different freezers*

Cooling rate: the cooling rate from initial temperature to crystallization temperature varied only between -34°C/min and -42°C in the TurboFreezer, while Conventional Freezer I showed a much higher variation. In Conventional Freezer II a rather slow cooling rate was measured, which is due to the program used with this freezer.

Plateau duration: the time needed to come back to T Crystallization after the temperature increase during the crystallization phase, varied from 40 seconds to 59 seconds in the TurboFreezer, while conventional freezers showed a higher variation depending on the location of the sensor. In Conventional Freezer I, the plateau duration varied from 15 to 61 seconds, in Conventional Freezer II from 32 to 79 seconds. A negative influence on homogeneity of semen quality after freezing and thawing can be suspected from such high variations. A quick removal of crystallization temperature has been reported to be beneficial to sperm quality.

The temperature at which crystallization took place was between -12°C and -15°C in the TurboFreezer Again, the variation in the two conventional freezers was higher: between -5°C and -15°C. T Crystallization of Conventional Freezer II and TurboFreezer were very similar, while Conventional Freezer I provided the highest variation in temperatures of crystallization.

The freezing rate between the end of the temperature plateau until reaching -80°C varied in Conventional Freezer I to double the value between the slowest and the fastest rate, depending on the location of the straw inside the freezer, and to even a much higher degree in Conventional Freezer II. It is nearly the same in the TurboFreezer at all measured locations.

## The following figures show the temperature profiles measured during trials with three freezers:

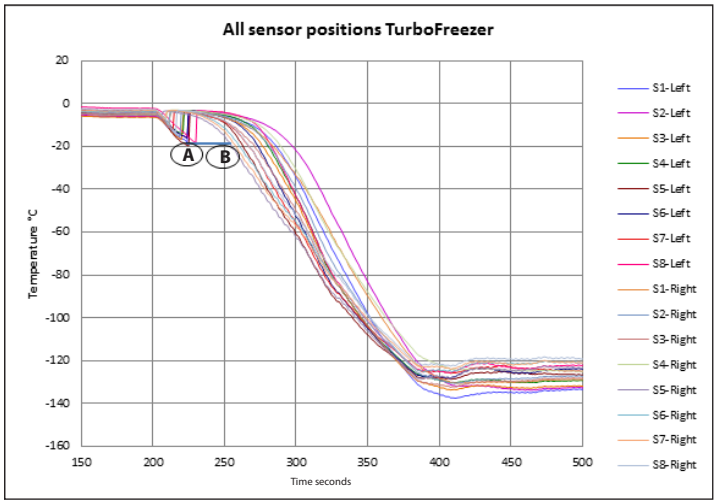



*Figure 6: Typical freezing curves of TurboFreezer, 16 sensors, 2 stacks (left and right)*

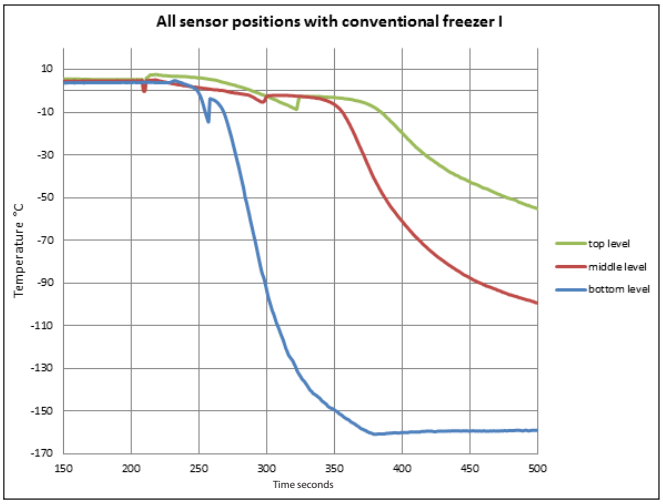



*Figure 7: Typical freezing curves of Conventional Freezer I, three sensors at three levels*

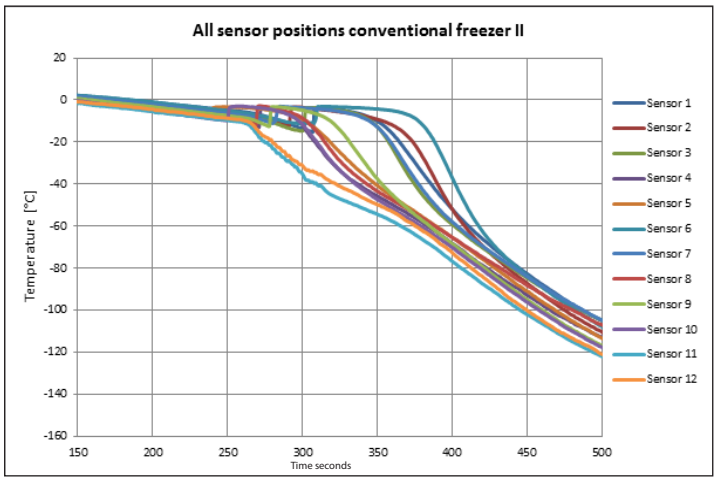



*Figure 8: Typical freezing curves of Conventional Freezer II, 12 sensors at three levels*

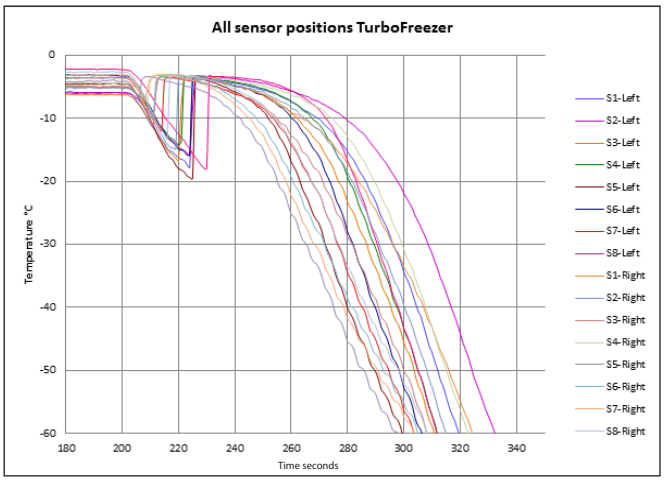



*Figure 9: Zoom on crystallisation phase and plateau duration in TurboFreezer*

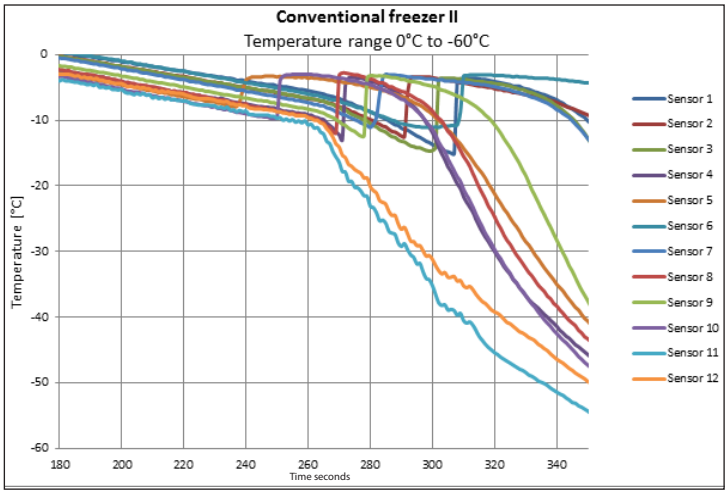



*Figure 10: Zoom on crystallization phase and plateau duration in Conventional Freezer II*

In summary, conventional freezers show unequal distribution and rates of temperature decrease. Also, the time needed to come back to the temperature after the increase originated by crystallization varies excessively depending on the location of the straw inside the freezer. Therefore, freezing is not performed in a uniform way for all straws in both conventional freezers.

It is concluded, that even though the TurboFreezer processes a very high number of racks per cycle, the freezing parameters are clearly improved in comparison to commonly used conventional freezers.

## Study 2

### Effect of homogeneous freezing temperature on post-thaw bull semen quality parameters

In a study performed by the University of Hannover in 2011, ejaculates from a total of 18 bulls were analyzed according to their position in the TurboFreezer during freezing. Figure 11 summarizes the results of the analysis regarding percentage of plasma membrane and acrosome intact sperm (PMAI), damaged acrosomes and motility (total and progressive) immediately after thawing. There is no statistically significant difference ($p<0.05$) between the different locations within the freezing chamber.

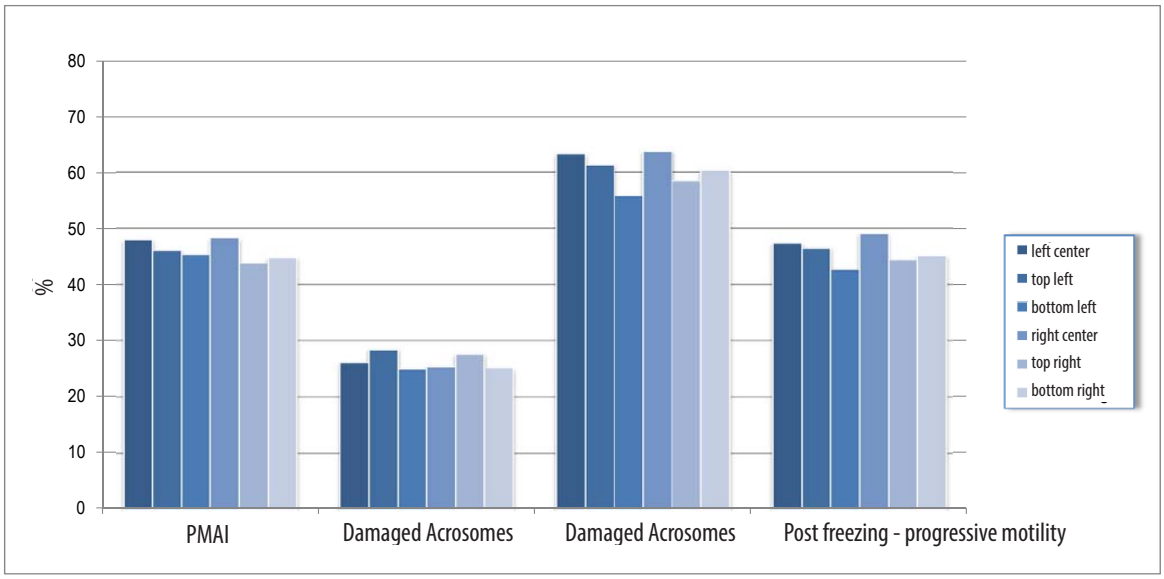



*Figure 11: Comparison of effect of different locations in TurboFreezer during freezing on post thaw semen quality (percentage of plasma membrane and acrosome intact sperm [PMAI], damaged acrosomes, post freezing total and progressive motility)*

## Study 3

### Influence of initial chamber temperature on sperm quality

The TurboFreezer offers a customized choice for the temperature at which the freezing process is started. This allows extremely high cooling rates. To detect the effect of this start temperature on the post-thaw bull semen quality, 18 ejaculates of 16 different bulls were frozen in a standard freezer and in the TurboFreezer and analyzed for semen quality parameters after thawing. Three different initial chamber temperatures were evaluated with the TurboFreezer: +5°C, -50°C and -120°C. The frozen thawed straws were analyzed for PMAI (plasma membrane and acrosome intact sperm), damaged acrosomes, total motility and progressive motility. PMAI and damaged acrosomes were evaluated with a flow cytometer with FITC-PNA and PI fluorescent stains. The thawing protocol and flow cytometer protocol were identical for all semen samples.

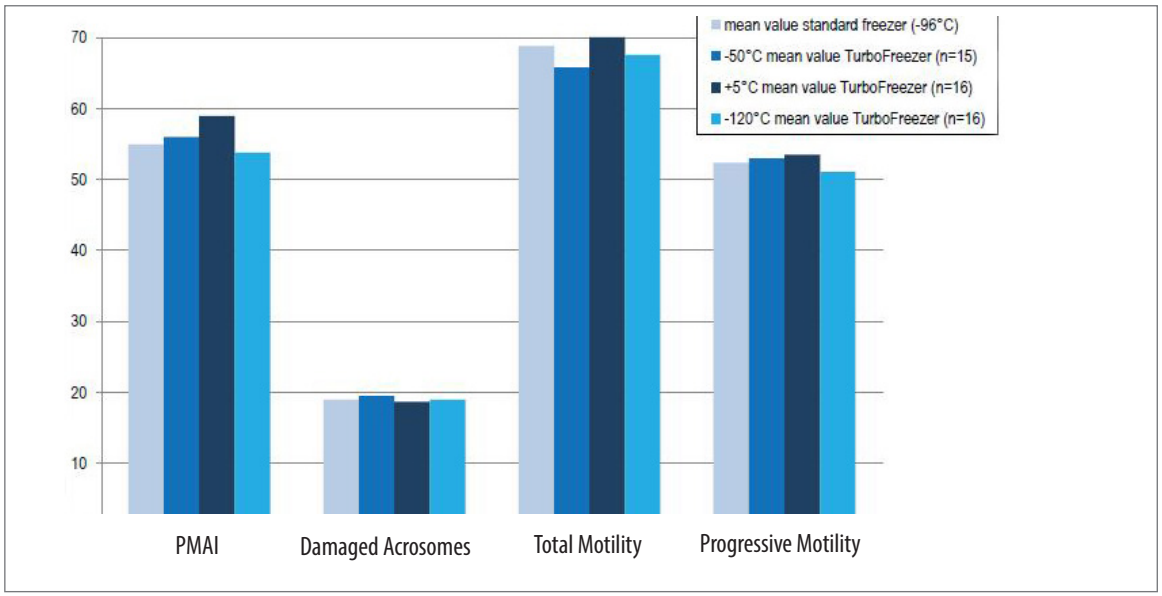



*Figure 13: Comparison of different start temperatures for freezing in the TurboFreezer on post-thaw semen quality*

Results indicate an advantage for a start temperature of +5°C for total motility, progressive motility and PMAI. The percentages of damaged acrosomes were identical for all start temperatures.

## Study 4

### Variation of plasma membrane integrity at different initial chamber temperatures

Single straws of 10 different bulls, frozen at different initial temperatures, were analyzed for plasma membrane and acrosome intact sperm (PMAI). The coefficient of variation was found to be much higher with the standard freezing method in comparison with the TurboFreezer (see figure 14). Freezing performed with the TurboFreezer leads to a more uniform quality of the semen.

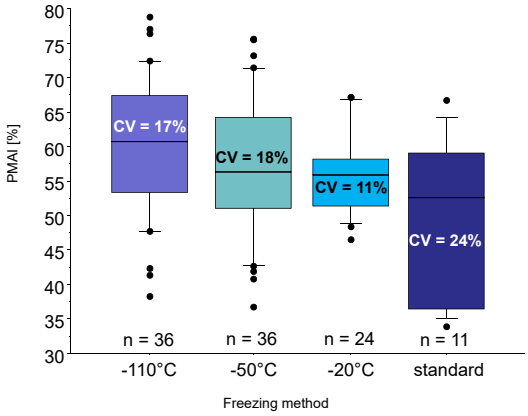



*Figure 14: Coefficient of variation for PMAI in post-thaw semen samples frozen with different start temperatures (-110°C, -50°C and -20°C) in TurboFreezer compared to standard freezer*

**In conclusion, the studies shown above prove the outstanding performance of the TurboFreezer with regard to uniformity of the freezing process and semen quality of every single straw. This is due to the unidirectional vapor flow provided by the innovative design.**