Establishment of a sperm bank for infertility treatment at Da Nang Hospital for Women and Children

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Abstract

Objectives: To evaluate the effectiveness of sperm bank for infertility treatment at Da Nang Hospital for Women and Children.

Materials and Methods: A cross-sectional descriptive study was conducted on 88 cryopreserved sperm samples at Da Nang Hospital for Women and Children from December 2020 to September 2023.

Results: There were 3 cases of sperm donation (3.4%) and 85 cases (96.6%) of autologous sperm retrieval for ICSI. In the sperm donation group, the mean age, pre-cryopreservation sperm concentration, and motility rate were 37.7 ± 4.9 years, 20.0 ± 10.6 million/ml, and 21.3 ± 10.1%, respectively. In the autologous sperm retrieval group, the mean age, pre-cryopreservation sperm concentration, and motility rate were 31.7 ± 4.8 years, 2.7 ± 1.8 million/ml, and 20.9 ± 8.7%, respectively. The cryopreservation duration for sperm samples in the sperm donation group and the autologous sperm retrieval group was 3.7 ± 0.6 months and 5.3 ± 3.9 months, respectively. 92.2% of the cryopreserved samples were used for ICSI with fertilization rates, implantation rates, and clinical pregnancy rates of 71.0%, 35.6%, and 48.0%, respectively.

Conclusions: Our research shows that autologous sperm cryopreservation is the most common practice in the sperm bank. The proportion of sperm donations made for humanitarian purposes remains relatively low. The utilization of cryopreserved sperm in ICSI procedures has demonstrated promising outcomes.

Keywords: donation, infertility, sperm bank.

1. INTRODUCTION

Sperm banks play a pivotal role in preserving male fertility, particularly in the era of assisted reproductive technologies (ART). The first sperm banks dedicated to infertility treatment were established in Iowa (USA) and Tokyo (Japan) in 1964. This is a significant scientific breakthrough, similar to the success of cryopreservation of sperm with glycerol in 1949 and the birth of the first baby from frozen sperm in 1953. In the US, over 10 million babies have been born from sperm donated by commercial sperm banks in the past 40 years [1]. However, the source of sperm donations for banks remains limited globally [2], [3].

Sperm cryopreservation has garnered considerable attention since the early 18th century. In 1776, Spallanzani reported a case of sperm preservation using snow. Successful cryopreservation of human sperm was first reported in 1973 and, when properly implemented, enables long-term storage of sperm [4].

The efficacy of cryopreserved sperm in treating obstructive azoospermia and severe oligozoospermia remains a subject of debate. Some studies have shown no difference in clinical pregnancy rates between cryopreserved sperm from the epididymis or testes compared to non-cryopreserved sperm. In contrast, other studies suggest that cryopreservation of sperm reduces clinical pregnancy and live birth rates. Therefore, the authors advocate for well-designed studies to evaluate the effectiveness of cryopreserved sperm obtained from testicular sperm retrieval [5], [6].

Da Nang Hospital for Women and Children, established in 2012, serves as the city's tertiary referral center for obstetrics and gynecology, providing care to patients not only in Da Nang but also in the Central Highlands and Central Coast regions. The hospital also functions as a training facility for Hue University of Medicine and Pharmacy and the Faculty of Medicine, University of Da Nang. The hospital has successfully implemented in vitro fertilization (IVF) and other ART procedures. However, a study titled “Current Status of Sperm and Oocyte Donation Management at Assisted Reproductive Centers in 2018” by a group of authors from Hanoi Medical University and the Central Obstetrics and Gynecology Hospital, involving 23 Assisted Reproductive Centers nationwide, revealed that Thanh Hoa Obstetrics and Gynecology Hospital and Da Nang Hospital for Women and Children were the only two institutions that did not perform sperm donation, only offering cryopreservation of sperm and embryos for their own patients [2].

Therefore, this study aims to evaluate the
effectiveness of sperm bank for infertility treatment at Da Nang Hospital for Women and Children

2. MATERIALS AND METHODS

2.1. Study population: This study included 88 cases of sperm cryopreservation performed at Da Nang Hospital for Women and Children from December 2020 to September 2023.

2.1.1. Inclusion Criteria
- Autologous Sperm (AS):
  + Individuals undergoing cryopreservation prior to medical treatment (cancer, immune disorders)
  + Individuals undergoing cryopreservation due to ejaculatory dysfunction before assisted reproductive technology (ART) procedures, such as intrauterine insemination (IUI) or in vitro fertilization (IVF)
  + Individuals undergoing sperm retrieval from the epididymis (PESA, MESA) or testes (TESA, TESE)
  + Individuals undergoing cryopreservation for personal reasons
  + Individuals with other reasons
- Donated Sperm for Humanitarian Purposes
  + Individuals voluntarily donating sperm to the sperm bank (Donated Sperm)
  + Individuals voluntarily donating sperm for exchange from the sperm bank (Exchange Sperm)
- Sperm Donor Criteria
  + No prior sperm donation
  + Voluntary sperm donation
  + Age 18 years or older and preferably under 45 years
  + No infectious diseases (Hepatitis B, C, syphilis, HIV)
  + No genetic and/or psychiatric disorders
  + No family history of genetic and/or psychiatric disorders
  + Sperm quality meets the donation criteria of Da Nang Hospital for Women and Children
    Concentration: ≥ 15 × 10⁶ sperm/ml
    Motility: Progressive motility (PR) ≥ 32%
    Normal morphology: ≥ 2%
    Understanding anonymous sperm donation
- Exclusion Criteria
  - Sperm donors and recipients with HIV, sexually transmitted infections (STIs) such as syphilis, gonorrhea, etc.

2.2. Study Design: Cross-sectional descriptive study with longitudinal follow-up

2.3. Study Procedures

Sperm Bank Sample Intake Procedure
Sperm Samples Sources
- Autologous Sperm (AS)
  - Sperm retrieved from the epididymis (PESA, MESA) or testes (TESA, TESE)
  - Sperm cryopreservation for personal reasons
  - Donated Sperm for Humanitarian Purposes

Sample Collection Procedure
- Sperm samples by Self-Ejaculation
  - Essential testing for hepatitis B, syphilis, tuberculosis, and HIV
  - Semen collection and analysis according to World Health Organization (WHO) standards
- Sperm samples by Surgical Retrieval
  - Surgical sperm retrieval involves aspirating sperm from the epididymis or performing testicular biopsy to obtain sperm for fertilization with oocytes using intracytoplasmic sperm injection (ICSI) in cases of azoospermia or ejaculatory dysfunction.
  - Donated and Exchanged Sperm
    After being consulted by the doctor and understanding the information and regulations for sperm donors at the DNHWC Sperm Bank, sperm donors will:
    - Undergo testing for infectious diseases (HIV/AIDS, syphilis, hepatitis B virus, hepatitis C virus), blood type, and complete blood count (CBC) (at the Department of Laboratory Medicine). Simultaneously, a semen sample will be collected for semen analysis (at the Department of Infertility). If the test results are negative for all the above diseases and the semen analysis meets the standards, the sperm donor will be instructed to undergo additional HIV confirmatory testing and karyotyping. They will also receive counseling and undergo procedures related to sperm donation.
    - Sperm donors will be scheduled to return to the Department of Infertility, Da Nang Hospital for Women and Children to provide a semen sample for cryopreservation (after 2 - 3 days of abstinence) following a negative HIV confirmatory test and normal karyotype. If the semen sample quality meets the standards, the sample will be cryopreserved and considered a cryopreserved sample and entered into the sperm bank for coding. If the sample quality does not meet the standards, medical personnel will provide counseling and discard the sample, while rescheduling the patient to the Department of Infertility on another day for resampling. If the return time exceeds 3 months from the initial blood test, testing for infectious diseases must be repeated.
    - Upon the availability of two cryopreserved sperm samples at the sperm bank and their proper coding, the sperm bank- Da Nang Hospital for Women and Children shall have full authority to utilize the sperm samples for treatment purposes as stipulated in Article 5 of Decree No. 10/2015/NĐCP.

Sperm Sample Management
- Coding Procedure for Sperm Samples
  - For donated and exchange samples that meet the
criteria for inclusion in the sperm bank, a unique sample identification code will be assigned in the sperm banking management software to ensure anonymity.

- For autologous sperm sample, the patient's code (assigned upon registration at the reception desk on the first floor) will serve as the sample identification code. Upon submission of the sperm sample, the sperm bank will provide the donor with a receipt confirming the submission of the sample to the sperm bank. If the donor voluntarily donates the sample to the sperm bank and the sample meets the criteria for inclusion in the sperm bank, it will be assigned a unique identification code in the sperm bank management software.

2.4. Statistical analysis
The SPSS 25.0 software was utilised for analysing the collected data. Percentages and frequencies were computed to report categorical data. To evaluate discrepancies between groups, “independent samples t-tests” or “Mann-Whitney U-tests” were utilised according to whether variables had normal or skewed distributions. “Chi-squared tests” or “Fisher’s exact tests” were utilised when comparing the frequency distributions of categorical data. If collected data had low expected frequencies (< 5), “Fisher’s exact tests” were applied. Univariate logistic regression analysis was utilised to identify confounders of clinical pregnancies. The criterion for statistical significance applied in the primary analysis was P < 0.05.

3. RESULTS
Between December 2020 and September 2023, the Sperm Bank at Da Nang Hospital for Women and Children received 88 sperm cryopreservation samples, with the following characteristics:

3.1. Age distribution of study subjects

Table 1. Distribution of study subjects by age group

<table>
<thead>
<tr>
<th>Age</th>
<th>Sperm donation group</th>
<th>Autologous surgical sperm retrieval group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>&lt; 40</td>
<td>1</td>
<td>33.3</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>2</td>
<td>66.7</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Mean ± SD
Sperm donation: 37.7 ± 4.9
Autologous surgical sperm retrieval: 31.7 ± 4.8

Median (95% CI)
Sperm donation: 40 (25.4 - 49.9)
Autologous surgical sperm retrieval: 31 (30.7 - 32.8)

For the sperm donation group, 66.7% were over 40 years old, with an average age of 37.7 ± 4.9 years, the youngest being 32 years old and the oldest being 41 years old.

For the self-sperm retrieval group, 94.1% were under 40 years old, with an average age of 31.7 ± 4.8 years, the youngest being 22 years old and the oldest being 47 years old.

3.2. Reason for sperm cryopreservation

Figure 1. Reason for sperm cryopreservation
There were 3 cases of sperm donation, accounting for 3.4%. Cases of autologous sperm retrieval and cryopreservation for ICSI accounted for 96.6%.
3.3. Surgical sperm retrieval methods

In 85 cases of surgical sperm retrieval, there was 1 case performed using the micro TESE method (1.2%), 3 cases of MESA (3.6%) and 81 cases of PESA (95.2%).

3.4. Characteristics of semen parameters before cryopreservation

Table 2. Semen parameters of the sperm donor group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Median (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm concentration (million/ml)</td>
<td>20.0 ± 10.6</td>
<td>16 (6.3 - 46.3)</td>
</tr>
<tr>
<td>Total sperm count (million)</td>
<td>55.0 ± 21.0</td>
<td>51.2 (3.4 - 107.3)</td>
</tr>
<tr>
<td>Progressive motility (%)</td>
<td>21.3 ± 10.1</td>
<td>20 (3.7 - 46.3)</td>
</tr>
<tr>
<td>Non-progressive motility (%)</td>
<td>23.3 ± 3.1</td>
<td>24 (15.7 - 30.9)</td>
</tr>
<tr>
<td>Vitality rate (%)</td>
<td>65.0 ± 13.2</td>
<td>70 (32.1 - 97.9)</td>
</tr>
<tr>
<td>Normal morphology (%)</td>
<td>2.7 ± 0.6</td>
<td>3 (1.2 - 4.1)</td>
</tr>
</tbody>
</table>

The average sperm concentration in the sperm donation group was 20.0 ± 10.6 million/ml. The average progressive motility rate was 21.3 ± 10.1%.

Table 3. Semen parameters before cryopreservation in autologous sperm retrieval group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Median (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm concentration (million/ml)</td>
<td>2.7 ± 1.8</td>
<td>2,2 (1.8 - 2.5)</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>20.9 ± 8.7</td>
<td>19 (19.0 - 22.8)</td>
</tr>
</tbody>
</table>

The average sperm concentration before cryopreservation was 2.7 ± 1.8 million/ml. The average motility rate was 20.9 ± 8.7%.

3.5. Cryopreservation Duration

Table 4. Cryopreservation duration

<table>
<thead>
<tr>
<th>Cryopreservation Duration (months)</th>
<th>Surgical sperm retrieval group n (%)</th>
<th>Sperm Donor Group n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6</td>
<td>53 (65.4)</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>6 - 12</td>
<td>21 (25.9)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>&gt; 12</td>
<td>7 (8.6)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>81 (100.0)</td>
<td>3 (100.0%)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>5.3 ± 3.9</td>
<td>3.7 ± 0.6</td>
</tr>
</tbody>
</table>

For surgical sperm retrieval group, the majority of sperm samples (65.4%) were cryopreserved for less than 6 months. The average cryopreservation duration was 5.3 ± 3.9 months.

For the sperm donation group, all sperm samples (100%) were cryopreserved for less than 6 months. The average cryopreservation duration 3.7 ± 0.6 months.
3.6. Utilization Status of Cryopreserved Sperm

Table 5. Sample utilization status

<table>
<thead>
<tr>
<th>Status</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Used</td>
<td>81</td>
<td>92.2</td>
</tr>
<tr>
<td>Awaiting Use</td>
<td>7</td>
<td>7.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>88</td>
<td>100</td>
</tr>
</tbody>
</table>

A high proportion (92.2%) of the cryopreserved sperm samples had been used for ART procedures.

3.7. Efficacy of using cryopreserved sperm retrieved from epididymis

Table 6. Outcomes of using cryopreserved sperm

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of embryos transferred</td>
<td>2.1 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Implantation rate</td>
<td>35.6 (53/149)</td>
<td></td>
</tr>
<tr>
<td>Clinical pregnancy rate</td>
<td>48.0 (47/98)</td>
<td></td>
</tr>
<tr>
<td>Multiple pregnancy rate</td>
<td>12.8 (6/47)</td>
<td></td>
</tr>
</tbody>
</table>

The total number of embryos transferred and the total embryo transfer cycles were 149 and 98, respectively. The total number of gestational sacs was 53.

Implantation rate, clinical pregnancy rate and multiple pregnancy rate were 35.6%, 48.0%, and 12.8%, respectively.

4. DISCUSSION

4.1. Age of the study subjects

In our study, the mean age in the sperm donation group was 37.7±4.9 years, with a minimum age of 32 years and a maximum age of 41 years. The mean age of participants in our study was higher than that reported in other studies. For instance, Gao et al. [7] observed an average age of 30.54 ± 4.44 years in a study involving 1559 sperm cryopreservation cases from sperm donors for ICSI procedures. Similarly, Barrera et al. [8] reported an average age of 23.90 ± 3.98 years in a study of 72 sperm donors. This difference may be due to cultural norms and different attitudes towards sperm donation between countries.

For the autologous surgical sperm retrieval group, the mean age in our study was 31.7±4.8 years, with a minimum age of 22 years and a maximum age of 47 years. This finding aligns with the results of other studies investigating autologous sperm retrieval procedures. For instance, Mai Bá Tiến Dũng (2021) reported an average age of 31.5 ± 4.79 years in a group of patients with azoospermia, with the youngest patient being 23 years old and the oldest being 48 years old [5]. Similarly, Yurci et al. [9] observed an average age of 30.4 ± 5.1 years in a group of patients with azoospermia who underwent ICSI with cryopreserved sperm. However, our findings contrast with those of Alfaraj et al. [10], who reported a higher average age of 40.0 ± 10.0 years in a group of patients with azoospermia. These variations in age distribution across different studies may be attributed to regional differences in marriage ages and, consequently, variations in the timing of disease diagnosis.

4.2. Cryopreservation reasons

In our study, cryopreservation was performed primarily for autologous surgical sperm retrieval (96.6%) with the intent to utilize the cryopreserved sperm for ICSI procedures. Sperm donation accounted for a small proportion of cases (3.4%).

Currently, there is a global shortage of sperm donors, including in Vietnam. According to a study by Nguyễn Thị Huyền Linh et al., approximately 1000 individuals seek oocyte donation and 700 seek sperm donation annually in Vietnam [2]. However, in 2017, only 518 and 596 individuals donated sperm and oocytes, respectively, across 21 assisted reproductive technology (ART) centers in the country [2].

A recent study (2022) by Pacey AA reported that among 11,712 individuals who registered to donate sperm on the Cryos website in the United States and Denmark, only 3.79% ultimately completed the donation process, had their sperm cryopreserved, and had their samples utilized [11]. The sperm donation acceptance rate in Denmark (6.53%) was significantly higher than in the United States (1.03%) [11]. In China, the acceptance rate is estimated to be around 3.2% [12]. These variations in sperm donation acceptance rates across different countries may be attributed to differences in cultural norms, customs, regulations, and cryopreserved sperm acceptance standards.

4.3. Sperm retrieval techniques

In our study, there were 85 cases of surgical sperm retrieval, of which, 1 case was performed using the micro TESE method (1.2%), 3 cases of MESA (3.6%)
and 81 cases of PESA (95.2%). For cases where PESA was unsuccessful, MESA was employed as a backup technique (3 cases). In the case of non-obstructive azoospermia, microTESE was utilized (1 case).

Until now, the optimal site for sperm retrieval, whether the epididymis or the testis, remains a topic of ongoing research. While both approaches have been successfully employed in ICSI procedures, the question of which technique yields superior outcomes persists. A study by Javed A et al. [13] investigated the comparative efficacy of fresh and cryopreserved sperm retrieved from the epididymis and testis in ICSI cycles. They included a total of 287 ICSI cycles involving 138 patients with obstructive azoospermia (OA) and 149 patients with non-obstructive azoospermia (NOA). The sperm retrieval methods employed were PESA (73 fresh samples, 65 cryopreserved samples) and TESE (128 fresh samples, 21 cryopreserved samples). The results of this study revealed no statistically significant difference in clinical pregnancy rates between fresh and cryopreserved sperm samples from either the epididymis or the testis (p > 0.05). For PESA, the clinical pregnancy rates were 36.5% for fresh samples and 34.6% for cryopreserved samples. Similarly, for TESE, the clinical pregnancy rates were 29.2% for fresh samples and 24.3% for cryopreserved samples [13].

Liu et al. conducted a meta-analysis in 2020, encompassing 20 studies, to evaluate the impact of using cryopreserved epididymal and testicular sperm on ICSI outcomes in patients with obstructive azoospermia [14]. The findings revealed a higher clinical pregnancy rate in the fresh epididymal sperm group (44.1%) compared to the cryopreserved epididymal sperm group (36.6%, p < 0.05). For testicular sperm, there was no statistically significant difference in clinical pregnancy rates between the fresh sperm group (47.8%) and the cryopreserved sperm group (38.2%, p>0.05). Additionally, no statistically significant difference was observed in clinical pregnancy rates between the cryopreserved epididymal sperm group (36.6%) and the cryopreserved testicular sperm group (38.2%, p>0.05). However, the authors acknowledged limitations in their analysis and emphasized the need for further research to definitively establish the efficacy of fresh vs. frozen epididymal and testicular sperm for treating obstructive azoospermia [14].

### 4.4. Efficacy of ICSI with cryopreserved sperm

In our study, we utilized 81 out of the 88 cryopreserved sperm samples available, accounting for 92.2%. This utilization rate highlights the feasibility and effectiveness of employing cryopreserved sperm in ICSI procedures. The average fertilization rate in our study was 71.0%. This finding aligns with the results of other studies that have employed cryopreserved epididymal sperm for ICSI, such as the work of Javed A (65.5%) [13] and Ou L et al. (75.67%) [15]. These comparable fertilization rates across studies suggest that cryopreservation does not significantly compromise the fertilizing potential of sperm.

The implantation rate in our study was 35.6%. This outcome is comparable to the findings of Vũ Thị Bích Loan, who reported an implantation rate of 37.1% using cryopreserved PESA sperm [16]. However, our implantation rate was higher than that observed by Nguyễn Thị Liên Hương, who reported an 18% implantation rate with cryopreserved PESA sperm [17]. Conversely, our implantation rate was lower than that reported by Lewin et al., who achieved an implantation rate of 59.7% [18]. Variations in implantation rates across studies may be attributed to a multitude of factors, including embryo quality, endometrial receptivity, maternal age, embryo transfer technique, the number of embryos transferred, and particularly, the quality of the embryos.

The clinical pregnancy rate in our study was 48.0%. This outcome is consistent with the findings of several other studies that have utilized cryopreserved PESA sperm for ICSI, including those by Nguyễn Thị Liên Hương (48.6%) [17], Trương Thị Thanh Bình (48.6%) [19], and Ou et al. (55.2%) [15]. However, our clinical pregnancy rate was higher than that reported by Javed A et al. (34.5%) [13] and Lewin J et al. (32.4%) [18], while it was lower than the rates achieved by Huang C et al. (70.51%) [20] and Yu X (62.76%) [21]. These variations in clinical pregnancy rates may be attributed to differences in patient selection criteria, laboratory protocols, and embryo assessment methods.

### 5. CONCLUSIONS

This study highlights the predominance of autologous sperm cryopreservation within the sperm bank. The proportion of humanitarian sperm donations remains limited. The use of cryopreserved sperm in ICSI procedures has demonstrated promising outcomes.

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