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ABSTRACT

Research Question

The reproductive potential of transgender people may be impaired by gender-affirming hormone treatment (GAHT) and is obviously suppressed by gender-affirming surgery involving bilateral orchiectomy. The evolution of medical support for transgender people has made fertility preservation strategies possible. Fertility preservation in transgender women mainly relies on sperm cryopreservation. There are few studies on this subject, and the sample sizes are small. Consequently, is fertility preservation procedure feasible and effective in trans women?

Design

In this retrospective study, the management of fertility preservation in transgender women referred to our centre for sperm cryopreservation was reported, and trans women's semen parameters were compared with sperm donors' semen parameters.

Results

Ninety-six per cent of transgender women who had not started treatment benefitted from sperm cryopreservation, compared to 80% of those who attempted a therapeutic window and 50% of those receiving hormonal treatment at the time of sperm collection. No major impairment of semen parameters was observed in transgender women who had not started GAHT compared to sperm donors. However, even though the frequency of oligozoospermia was not different, two transgender women presented azoospermia. Some transgender women who had started GAHT could benefit from sperm freezing. None of them was treated with GnRH analogues.

Conclusions

Parenthood strategies for transgender people have long been ignored. Nevertheless, this issue is important to consider, especially since medical treatments and surgeries may be undertaken in adolescents or very young adults. Fertility preservation should ideally be offered prior to initiation of GAHT.

KEY WORDS

Fertility preservation; sperm cryopreservation; semen parameters; transidentity; transgender woman

INTRODUCTION

Trans identity is defined as a gender identity different from the sex assigned at birth. The evolution of transgender people generally corresponds to a physical transition that may involve several stages, including changes in physical appearance and clothing style, as well as medical and surgical interventions. Medical treatments include hormone therapy that suppresses the sexual characteristics of the original gender, usually combined with gender-affirming hormone treatment (GAHT), which induces the evolution of physical characteristics towards the gender with which the individual identifies (Tangpricha and den Heijer, 2017). For transgender women, hormonal treatment generally consists of the administration of hormones (anti-androgens and progestogens with or without oestrogens) that allow feminisation. Gender-affirming surgery, although not systematic, is also a therapeutic option.

The reproductive potential of transgender people is suppressed by gender-affirming surgery involving bilateral orchiectomy, and it can also be impaired by hormonal treatments. Although the consequences likely vary widely based on the treatment strategy (Schneider et al., 2015), testicular histology of transgender women shows a major decrease in or even a lack of spermatogenesis after initiation of GAHT (Schneider et al., 2017). Moreover, there is little research on the potential recovery of normal spermatogenesis during a therapeutic window, and discontinuing GAHT is usually difficult for transgender people to consider because it is viewed as a step backwards. Hence, offering fertility preservation prior to the initiation of hormonal treatment is of particular importance. Numerous scientific organisations, including the American Society of Reproductive Medicine (ASRM), have proposed recommendations concerning information about the potential impact of treatments on reproductive functions and about fertility preservation techniques in a trans identity context (W-PATH, Ethics Committee of the American Society for Reproductive, 2015).

The main method of fertility preservation for transgender women is sperm cryopreservation. There are few studies on transgender women who have benefitted from sperm cryopreservation, and the sample sizes are small (Baram et al., 2019). A significant alteration in semen parameters was observed in transgender women taking GAHT, as well as a high risk of azoospermia (Adeleye et al., 2019). However, a recent retrospective study showed that the sperm parameters of transgender women before beginning GAHT were significantly lower compared to WHO data from the general population and nearly 10% of them presented with azoospermia (de Nie et al., 2020).

Although the need to inform patients about the effect of the transition on fertility and the options for fertility preservation has been emphasised, the suspected sperm alterations could complicate and limit the efficiency of such procedures. In this study, we aimed to evaluate the feasibility and effectiveness of the fertility preservation procedure in transgender women by comparing their semen parameters with those of a population of healthy sperm donors and by reporting the results of the cryopreservation.

MATERIALS AND METHODS

Patient selection

This is a retrospective study of transgender women who contacted the Assisted Reproductive Technology (ART) centre at Tenon University Hospital, Paris, between 2018 and 2020. They contacted the ART centre to get information about fertility preservation and, in most cases, to benefit from sperm cryoconservation. Most of the patients were referred by their physicians (endocrinologists or general practitioners). A medical prescription was necessary for them to benefit from sperm freezing. Patients were offered medical and psychological consultations, followed, if necessary, by an appointment for sperm collection and freezing.

Data collection

The health care path of transgender women after the first contact in our centre was fully recorded, including attendance to the different appointments, such as consultations and sperm freezing. Data about age, hormonal treatment, semen parameters and sperm cryoconservation were collected. The patients were classified into three groups based on GAHT intake: no history of hormonal medication (NHM), previous hormonal medication (PHM) and current hormonal medication (HM).

Clinical and biological data about sperm donors recruited in our public centre between 2018 and 2020 were also collected in order to compare semen parameters between the groups. The sperm donors (SD) were healthy men aged 18 to 44 years old, with or without children. All sperm donors presented normal semen parameters.

Our study protocol was approved by a local ethics committee (IRB CLEA-2020-109) on 17th April 2020.

Sperm parameters analysis

Semen samples were collected following masturbation into a sterile plastic cup in the laboratory. After 30 minutes of liquefaction at room temperature, conventional semen parameters (semen volume and sperm concentration and motility) were evaluated according to WHO guidelines (WHO, 2010). Sperm morphology was assessed using David’s criteria (Auger et al., 2016). Oligozoospermia, or a decrease in total sperm count, is defined as a total of fewer than 39 million spermatozoa in the ejaculate according to WHO guidelines (WHO, 2010).

Only semen parameters from the first sperm collection were considered for statistical analyses. All samples from patients and sperm donors were collected at the same centre and analysed under the same conditions.

Sperm freezing

The semen samples of trans women and sperm donors were frozen according to the same standardised protocol. The semen samples were diluted with cryoprotectant medium (SpermFreez^{TM}, FertiProNV, Belgium) and distributed into straws (CBS^{TM} High Security Sperm 0.3ml Straw, CryoBioSystem, Group IMV Technologies). They were frozen in liquid nitrogen vapours using an automatic freezer (Nano-Digitcool, CryoBioSystem, Group IMV Technologies). The straws were then plunged into liquid nitrogen and stored in nitrogen tanks. Freezing tolerance was evaluated after one straw was thawed. Motility and sperm concentration were analysed, and the total number of progressive motile spermatozoa per straw (NMSPS) was calculated. A possible assisted reproductive technology strategy was defined according to NMSPS as follows: straws containing less than one million progressive motile sperm were considered usable for *in vitro* fertilisation with (ICSI) or without (IVF) intracytoplasmic sperm injection, and straws containing more than one million progressive motile sperm were considered usable for intrauterine insemination (IUI).

Statistical analysis

Semen parameters were compared between transgender women who had not started GAHT and sperm donors, as well as between transgender women who had started GAHT and those who had not. The number of sperm collection appointments was also compared across the three groups of trans women.

Variables are presented as mean ± standard error of measurement (SEM) for quantitative variables and as a percentage for qualitative variables. Quantitative variables were analysed using an independent *t*-test or Wilcoxon-Mann-Whitney test when appropriate and Fisher's exact test for qualitative variables. All statistical analyses were performed using Prism 6 Software (GraphPad Software Inc., La Jolla, CA, USA), and $p < 0.05$ was considered significant.

RESULTS

Description of the population

Between June 2018 and November 2020, 118 fertility preservation counselling appointments were proposed. Twenty-two patients cancelled the appointment and 96 patients were seen in medical and psychological appointments. Among them, 83 attempted at least one sperm collection. One patient experienced sperm collection failure, nine presented with azoospermia and 73 could benefit from sperm cryopreservation (**Figure 1**).

Among the 82 patients for whom sperm parameters could be evaluated, 65 patients had not started GAHT (NHM), five patients declared they had stopped treatment three to six months before sperm collection (PHM) and twelve patients were still on hormonal medication (HM) (**Table 1**).

Comparison between trans women with no history of GAHT (NHM) and sperm donors

The transgender women were significantly younger than the sperm donors (23.9 ± 0.6; (NHM), 27.2 ± 2.5 (PHM), 30.8 ± 3.1 (HM) vs 35.1 ± 1.0 (SD); $p<0.01$). The main semen parameters, including semen volume, sperm concentration, progressive motility and vitality, were not statistically different between transgender women and sperm donors (**Table 1**). However, normal sperm morphology was significantly lower in transgender women than in sperm donors ($p=0.004$). Although two transgender women presented with azoospermia, the transgender women did not display a higher frequency of oligozoospermia compared to the sperm donors (**Table 1**).

Comparison between trans women with no history of GAHT (NHM) and trans women with a history of GAHT (PHM and HM)

The patients with current hormonal medication (HM) were significantly older than the women with no history of GAHT (NHM) ($p<0.01$). All semen parameters (volume, concentration, motility and morphology) were significantly altered in patients with current hormonal medication in comparison with those who never had hormonal treatment ($p=0.04$, $p<0.01$, $p=0.01$, $p<0.01$, respectively). The finding of oligozoospermia and azoospermia was also more frequent ($p<0.01$) (**Table 1**). No differences were observed in transgender women who stopped hormonal medication before sperm cryopreservation, but the number of patients included in the study was low.

Results of trans women's sperm cryopreservation

Ninety-seven per cent of women who had not started treatment benefitted from sperm cryopreservation, compared to 80% of those who attempted a therapeutic window and 50% of those receiving hormonal treatment at the time of sperm collection (**table 2**). The total number of progressive motile spermatozoa per straw (NMSPS) was not significantly different across the three groups, but progressive motility after thawing was reduced in women under GAHT compared to women with no history of hormonal medication (p=0.03). Possible ART strategies (IUI vs IVF/ICSI) were not different between groups.

The majority of the transgender women who had not started treatment (76.2%) and those who were under hormonal treatment (66.7%) visited the centre only once for semen collection and freezing, while most patients who attempted a therapeutic window (75.0%) had to visit at least twice.

The patients under GAHT therapy who could benefit from sperm cryopreservation were using oestrogens combined with progesterone (n=5) or oestrogens combined with spironolactone (n=1). None of the patients taking cyproterone acetate (alone or associated with oestrogens) (n=6) could benefit from sperm cryopreservation due to azoospermia.

DISCUSSION

This study represents the first large French case series of transgender women referred for sperm cryopreservation for fertility preservation purposes. No major impairment of semen parameters was observed in transgender women who had not started GAHT compared to sperm donors. We observed an increase in morphological abnormalities in transgender women, but the clinical consequences are likely irrelevant (Gatimel et al., 2017). Moreover, even though the frequency of oligozoospermia did not seem different, two transgender women presented with azoospermia in our case series of 65, corresponding to an unexpectedly high prevalence. These findings are in line with previous publications suggesting that trans women had slightly poorer sperm parameters than cisgender men (Li et al., 2018) or young fathers (Marsh et al., 2019) or significantly decreased sperm parameters compared to WHO data from the general population (de Nie et al., 2020). The observed alterations may have been caused by an increase in scrotal temperature due to tight clothing or the tucking technique that hides the penis and testes (Thonneau et al., 1998). A decrease in the frequency or even the absence of ejaculation could also cause decreased sperm production (AlAwaqi and Hammadeh, 2017).

Some of the transgender women who had started treatment could benefit from sperm freezing. None of them were treated with cyproterone acetate, a treatment that led to azoospermia in 100% of the cases in our series. Although the literature on the subject remains scarce, a significant alteration in semen parameters was previously observed in transgender women treated with GAHT, as well as a high risk of azoospermia (Adeleye et al., 2019). In that study, the only patient with normal semen parameters was supplemented with oestrogens only (Adeleye et al., 2019). However, even if sperm production is maintained, there are concerns regarding the potential impact of hormonal treatments on the quality of the spermatozoa, such as epigenetic marks (Semet et al., 2017), as well as the safety of utilisation in terms of embryo development and child health.

Some of the transgender women included in our study discontinued their hormonal treatments during a therapeutic window of three to six months for fertility preservation purposes, either by their own decision or following medical advice. Our results suggest that the cyproterone acetate effects on semen parameters are not completely reversible. Many questions about the reversibility of GAHT remain, and the required duration of a therapeutic window for the recovery of normal spermatogenesis is unknown. Although it is likely to be at least three months (one complete cycle of spermatogenesis) (Barnard et al., 2019), it would probably depend on the duration, dose and nature of the hormones, as well as on individual factors. In a prior study, patients underwent a therapeutic window of three to six months and exhibited slightly poorer semen parameters than transgender women who had never taken hormones (Adeleye et al., 2019). In a different case, an absence of sperm production was described four months after treatment interruption (Barnard et al., 2019), suggesting that a complete reversal of GAHT-related semen impairment cannot be guaranteed. Moreover, a therapeutic window could be difficult to consider for people who have been in treatment for a long time because discontinuation of the treatment can lead to significant physical and psychological changes. Hence, offering fertility preservation prior to treatment initiation is of particular importance.

However, transgender patients may experience difficulties in accessing fertility preservation procedures. Fertility preservation for transgender patients is not equally available in all countries and regions, and it has been reported that information about fertility preservation remains unsystematic (Vyas et al., 2020). Health professionals need more comprehensive information in order to provide more information to patients and health care facilities. Moreover, although the costs of fertility preservation strategies are covered by national health insurance in France, and all patients have equal access to care, the cost of sperm banking can constitute a barrier in many other countries.

Parenthood strategies for transgender people have long been ignored. Nevertheless, this issue is important to consider, especially since medical treatments and surgeries may be undertaken in adolescents or very young adults (Rafferty et al., 2018). Although transgender women's fertility was not initially a priority, the recent increase in literature reflects a growing interest in this issue. In particular, some studies relying on questionnaires reveal that information about reproductive functions and fertility preservation opportunities is more and more systematic during the transition process (Baram et al., 2019). The majority of transgender men and women interviewed stated that they wanted to become parents, but few of them actually benefitted from fertility preservation techniques (Riggs and Bartholomaeus, 2018, Chen et al., 2019, Segev-Becker et al., 2020). This may be due to several factors. Fertility preservation procedures are sometimes responsible for a delay in treatment initiation. The cost may also be a hindrance in countries where patients have to pay for fertility preservation procedures. The possibilities of further use of cryopreserved gametes, depending on sexual orientation and the possibility of a partner carrying a pregnancy, may also play a role in the decision. Finally, it is also reported that transgender patients are sometimes not particularly attached to biological parenthood and would be open to alternative strategies such as adoption (Chen et al., 2019).

Frozen-thawed sperm can be used for intrauterine insemination (IUI) or *in vitro* fertilisation (IVF) with or without microinjection (ICSI). Very few cases of the utilisation of frozen sperm samples have been reported in the literature. In 2014, a child was born after IUI was performed with cryopreserved sperm in a couple consisting of a transgender woman and a cisgender woman (Wierckx et al., 2012). In 2017, a live birth was achieved following IVF using cryopreserved spermatozoa (Broughton and Omurtag, 2017), as well as an ongoing pregnancy following IVF with ICSI (Jones et al., 2016). French legislation does not allow the use of cryopreserved spermatozoa once the civil status change is official. To date, no request for cryopreserved sperm use has been made in our centre.

This study presents inherent limitations due to its retrospective design. However, it represents the first French case series from a reference centre for transgender care, and the number of patients included is relatively high compared to most published studies. Moreover, our studied population includes transgender women who had not started GAHT as well as transgender women who had started GAHT with and without a therapeutic window. Lastly, we were able to compare the semen parameters of these three groups with the semen parameters of a reference group of healthy sperm donors.

CONCLUSION

Although further use of cryopreserved gametes remains uncertain and will depend on current regulations in various countries, the cryopreservation of gametes represents an important step in global care for transgender people. Our research shows that it is feasible and effective to provide fertility preservation for trans women through sperm cryopreservation. When performed before the introduction of hormonal therapy, sperm parameters seemed slightly altered compared to those of healthy sperm donors. In this situation, one or two appointments were sufficient in most cases to obtain satisfactory results with a reasonable number of usable straws. Our results reveal that information about fertility preservation options should be provided early during the transition in order to facilitate optimal care and avoid the need to resort to a therapeutic window. The spread of this information will rely on networking between practitioners in endocrinology, surgery, gynaecology, reproductive biology, psychiatry and psychology.

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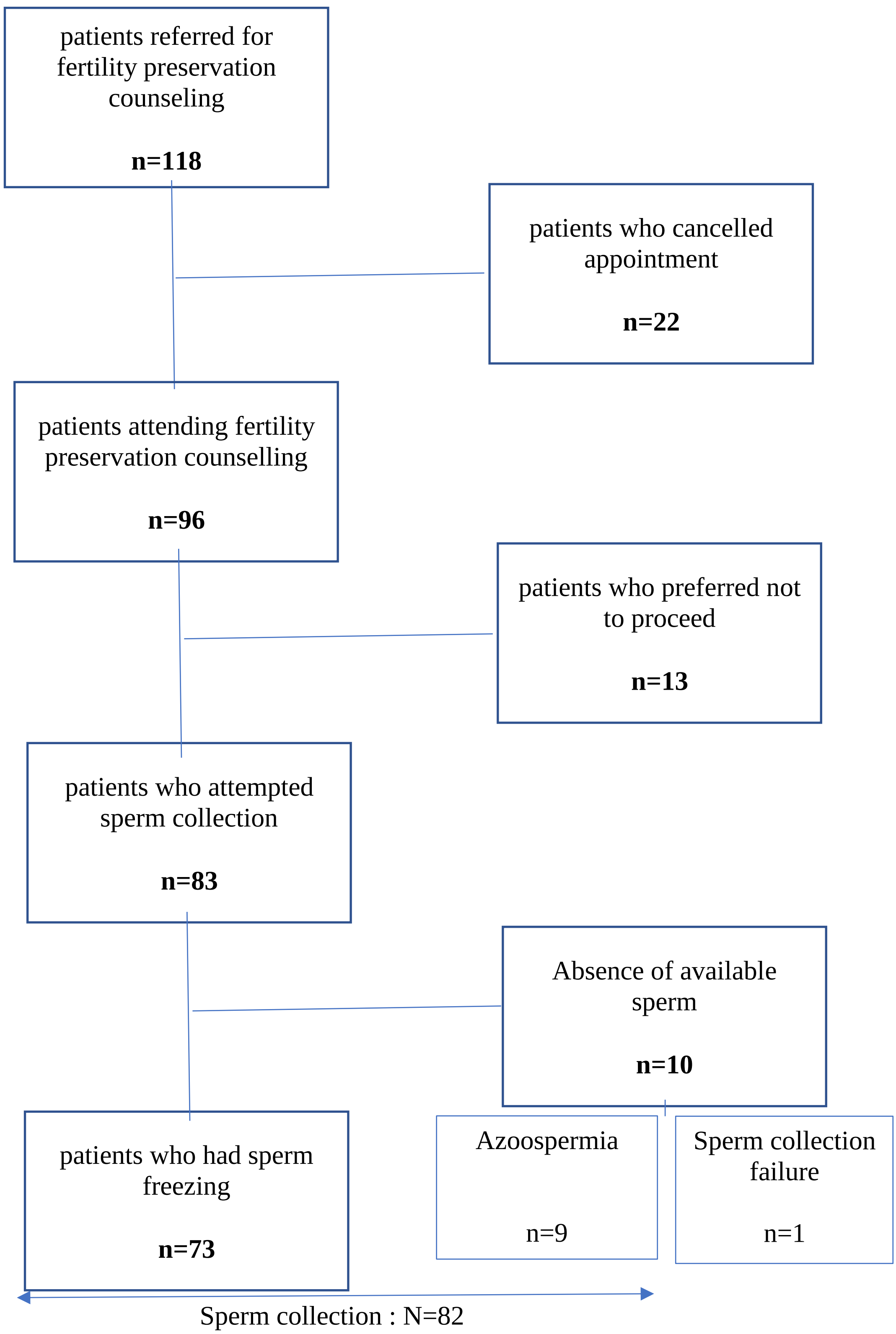

Figure 1. Studied population flow chart.

	Trans women						Sperm donor (SD)
	current hormonal medication (HM)	HM vs NHM p	previous hormonal medication (PHM)	PHM vs NHM p	no history of hormonal medication (NHM)	NHM vs SD p	
N	12		5		65		38
Age (years)	30.8 ± 3.1	**0.003**	27.2 ± 2.5	0.188	23.9 ± 0.6	<0.001	35.1 ± 1.0
Abstinence (days)	13.1 ± 4.4	0.072	5.8 ± 1.7	0.526	5.2 ± 0.7	**0.051**	3.4 ± 0.3
Volume (ml)	2.1 ± 0.6	**0.040**	3.3 ± 0.9	0.899	3.6 ± 0.2	0.932	3.6 ± 0.3
Sperm concentration (10^6.ml)	20.3 ± 9.6	**<0.001**	48.5 ± 28.4	0.382	62.3 ± 5.7	0.152	79.5 ± 12.5
Sperm numeration (10^6)	77.4 ± 42.1	**<0.001**	237.62 ± 155.0	0.475	214.8 ± 24.7	0.221	273.4 ± 46.6
Progressive mobility (%)	24.5 ± 8.2	**0.010**	26.6 ± 12.2	0.186	44.0 ± 1.7	0.054	42.9 ± 1.9
Vitality (%)	38.1 ± 9.6	**0.034**	32.7 ± 19.1	0.393	55.7 ± 1.9	0.062	61.3 ± 2.1
Morphology (%)	1.9 ± 0.6	**<0.001**	5.6 ± 3.6	0.337	7.5 ± 0.6	**0.004**	11.1 ± 1.2
Azoospermia n (%)	6 (50%)	/	1 (20%)	/	2 (3.1%)	/	0
Oligozoospermia n (%)	2 (16.7%)	/	2 (40%)	/	10 (15.4%)	/	4 (10.5%)
Azoospermia + oligozoospermia n (%)	8 (66.7%)	**0.002**	3 (60%)	0.062	12 (18.5%)	0.400	4 (10.5%)

Table 1: Conventional semen parameter values (Mean ± SEM). $p < 0.05$ was considered significant

		current hormonal medication (HM)	HM vs NHM p	previous hormonal medication (PHM)	PHM vs NHM p	no history of hormonal medication (NHM)
Total number of patients		12		5		65
N MtF who had sperm cryoconservation		6 (50%)	**<0.001**	4 (80%)	0.197	63 (96,9%)
Number of straws at first sperm collection		14.2 ± 3.1	0.249	13.7 ± 4.6	0.969	15.4 ± 0.7
NMSPS (10^6)		1.0 ± 0.4	0.102	4.5 ± 4.0	0.770	2.7 ± 0.3
Progressive mobility after thawing (%)		15.0 ± 4.6	**0.030**	34.7 ± 15.6	0.455	28.4 ± 1.9
Possible ART strategy N (%)	**IUI + IVF/ICSI**	3 (50%)	0.390	2 (50%)	0.592	43 (68.3%)
	IVF/ICSI	3 (50%)		2 (50%)		20 (31.7%)
Number of sperm collections		1.7 ± 0.3	0.196	2.8 ± 1.0	**0.015**	1.3 ± 0.1
Number of patients who visited once (%)		4 (66.7%)	0.630	1 (25%)	0.056	48 (76.2%)
Total straw number		15.7 ± 3.3	0.114	24.7 ± 5.5	0.500	17.8 ± 0.7

Table 2 : MtF sperm cryoconservation characteristics (Mean ± SEM). $p < 0.05$ was considered significant.

Abbreviations: MtF: male to female patients; ART: assisted reproductive technology; ICSI : intracytoplasmic sperm injection; IUI : intrauterine insemination; IVF: *in vitro* fertilization; NMSPS : number of progressive motile spermatozoa per straw ;