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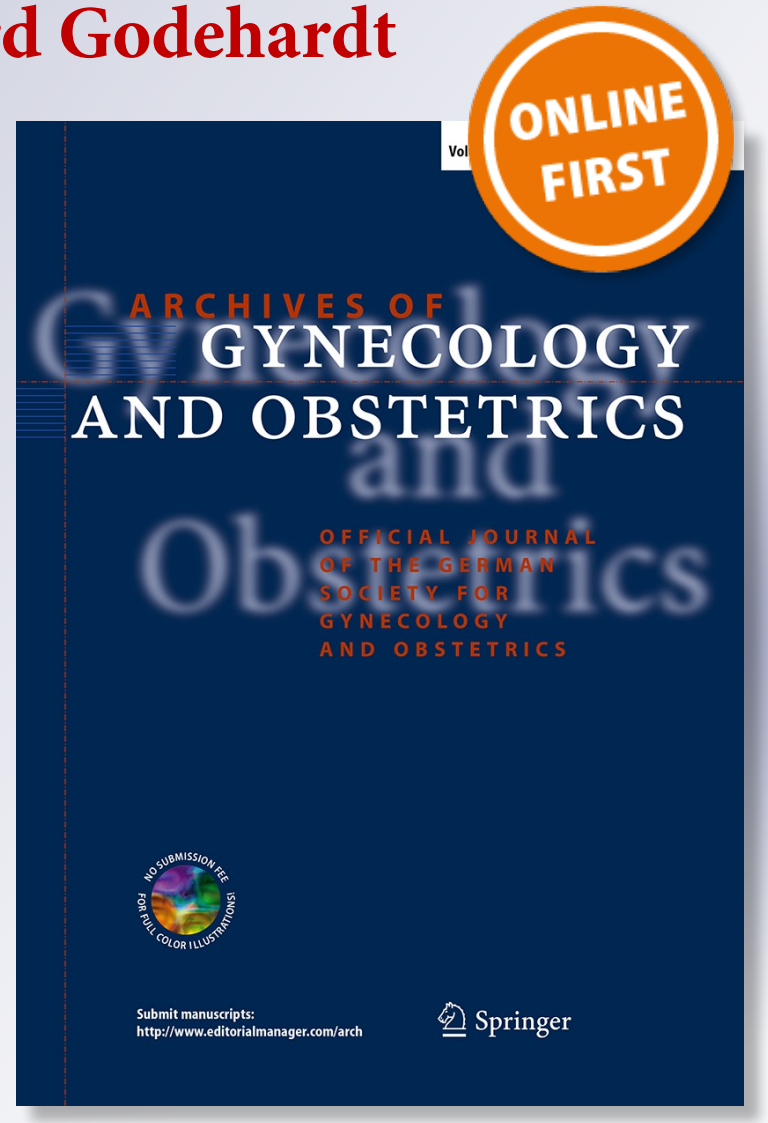

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GYNECOLOGIC ENDOCRINOLOGY AND REPRODUCTIVE MEDICINE

Impact of sperm cell source on the results of intracytoplasmic sperm injection

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Abstract
Purpose There is an ongoing debate whether the source of sperm cells, the etiology or the extent of male factor infertility has influence on the outcome of ICSI cycles.
Methods The results of intracytoplasmic sperm injection (ICSI) according to the source of spermatozoa in patients with severe male factor infertility were compared in a retrospective study: 249 couples underwent a total of 337 fresh ICSI cycles with the use of fresh motile testicular or fresh motile ejaculated spermatozoa.
Results For all variables, there were no statistically significant differences in the ICSI results between both groups. Fertilization rates were 46.8 % for testicular and 47.6 % for ejaculated spermatozoa. Live birth rates per embryo transfer were 20.4 % using testicular spermatozoa and 22.8 % using ejaculated spermatozoa.
Conclusions Neither the source of spermatozoa nor the etiology of severe male infertility has relevant impact on the results of ICSI cycles as long as fresh motile, morphologically normal spermatozoa are used. Therefore, in case of cryptozoospermia, we recommend to preferentially use ejaculated spermatozoa to prevent those men from an unnecessary testicular biopsy avoiding risks and costs implied.

Keywords Male factor infertility · Azoospermia · Cryptozoospermia · Testicular sperm extraction (TESE) · Intracytoplasmic sperm injection (ICSI) · Sperm cell source

Introduction

Matter of an intensive debate still is whether the source of sperm cells, the etiology or the extent of male factor infertility has influence on the outcome of ICSI cycles [1–3]. Even in cases with dominating teratozoospermia, negative effects on fertilization rate and pregnancy outcome after ICSI are questioned [4].

Cryptozoospermia is defined as spermatozoa absent in native semen samples but observed in a centrifuged pellet. It is unclear whether extracted testicular spermatozoa or ejaculated spermatozoa should be preferably used for ICSI in cryptozoospermia men [5–11]. Due to the assumed high spermatozoal sensitivity in cryptozoospermia men to the exposure to reactive oxygen species, inflammatory proteins and present leucocytes in the epididymis during the epididymal storage and passage [12–16] causing a higher rate of DNA damage, testicular sperm extraction (TESE) is sometimes preferred in men with cryptozoospermia [17]. A disadvantage of this procedure, besides the necessity of a

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surgical procedure, is that incompletely matured testicular spermatozoa may be selected for intracytoplasmic sperm injection [18, 19]. There is a suggestion that maturation state of testicular sperm cells may influence embryo viability and therefore implantation rate [18, 20]. However, this data is still very limited and the number of patients in these studies is rather low.

Because of this important, yet unresolved debate, we executed a retrospective data analysis of all ART (assisted reproductive techniques) cycles performed at green-ivf (Grevenbroich Endocrinology and IVF Center) from 1999 to 2008. We compared the outcome of ICSI according to the source of sperm cells and evaluated whether the use of either source had significant advantages in men with heavily impaired fertility.

Materials and methods

All ICSI cycles performed in a decade's time (1999–2008, 3,011 couples with a total of 8,048 ART cycles) were screened for ICSI cycles performed due to severely diminished male fertility (azoospermia and cryptozoospermia with well below than one million spermatozoa per ml in a centrifuged pellet of the native sperm sample). This provided a sample of 249 couples with a total of 337 ICSI cycles, divided into two cohorts of 60 ICSI cycles using fresh motile testicular spermatozoa (57 couples, FMTS group) and 277 ICSI cycles using fresh motile ejaculated spermatozoa (192 couples, FMES group). Couples remained within their initial cohort for duration of study.

For this case control study focusing on the impact of sperm source on ICSI results, we adjusted both groups for relevant co-factors. We only included couples with

- female age below 45 years of age and
- excluded couples with additional relevant female co-factors of subfertility (oligo-/amenorrhea, severe endometriosis, days 3–5 FSH $>$ 10mIU/ml and/or AMH $<$ 0.5 ng/ml, uterine factors; couples with additional tubal subfertility or only minimal endometriosis were permitted).

For 57 men of the FMTS group and for 192 men from the FMES group, histological results of testicular biopsies were available. Based on the results of these histological examinations, the medical history and the clinical examination, the men of each group could be classified into the different diagnostic sub-groups of male infertility (Figs. 1, 2).

This study was conducted in accordance with the principles of the Declaration of Helsinki. All couples signed an informed consent concerning data storage and the reporting and transfer of anonymised results to the National IVF Register.

Sperm recovery

Fresh testicular biopsies were washed with SpermRinse™, mechanically fragmented and examined for the presence of motile sperm cells. One testicular biopsy was set aside for histological and pathological examination. Supernumerary testicular biopsies were frozen if sufficient motile sperm cells were collected for the ICSI procedure.

In cases of cryptozoospermia, the native ejaculate was covered with 1.5 ml SpermRinse™, homogenized and centrifuged repeatedly.

Intracytoplasmic sperm injection

Standard ICSI procedure was applied using only motile, morphologically normal sperm cells. Intracytoplasmic morphologically selected sperm injection (IMSI), described as a specific method of sperm selection using high-magnification microscopy, was not applied. According to the German Embryo Protection Act, a maximum of 2–3 zygotes were cultured and supernumerary fertilized oocytes cryopreserved.

Embryo transfer was preferentially performed on day 3, in some cycles on day 2 for non-medical reasons. According to the German Embryo Protection Act of 1990, we refrained from selecting the best embryos for transfer.

Twelve to thirteen days after, transfer pregnancy tests were run. A week after that, ultrasonography was performed to diagnose a clinical pregnancy (visible gestational sac) and an ongoing pregnancy (visible embryonal heartbeats) 10–14 days thereafter. Within 3–6 months after calculated day of delivery, a follow-up telephone call was done.

Medical and laboratory data were recorded using the clinic management program MEDISTAR, the IVF laboratory managing program RECDATE and Microsoft EXCEL. Data were analyzed using the SAS package, version 9 (SAS Institute Inc., Cary, USA). According to the source of the sperm cells, rates of fertilization, implantation, pregnancy, live births and abortions were calculated and statistically analyzed. For categorical data, the Exact-Fisher Test and for quantitative data, the Student's t test for unequal variances were used. The results were stratified according to different age classes of treated women as being one of the most important factors of success. Due to the retrospective nature of this cohort study, we did not adjust for multiple testing. P value below 0.05 is reported as significant.

Results

For all men in the FMTS group ($n = 57$), a diagnosis of the etiology was made based on both, histopathological finding

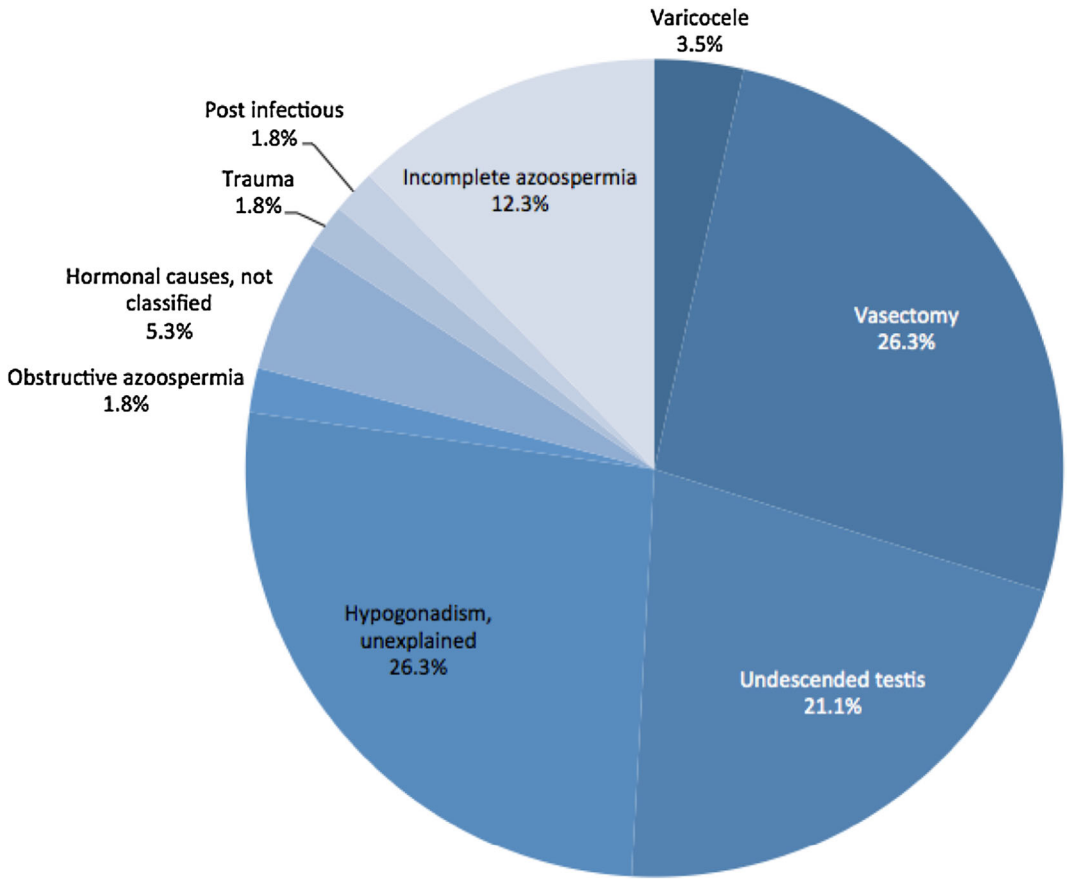

Fig. 1 Different etiologies of infertility in men with a azoospermia (testicular sperm cell group, FMTS)

and clinical/endocrinological examination (Fig. 1): 26.3 % of all azoospermic men undergoing testicular biopsy had a previous vasectomy, 26.3 % suffered from an unexplained hypogonadism with decreased size of testicles and a complete azoospermia in the native ejaculate and the pellet even after extended search. FSH and testosterone levels were within a normal range in the majority of these cases; sound karyotypes were detected and medical history analysis was negative for speculative reasons for severe male infertility (i.e., previous genital or severe general infections, preterm labor, malignant disease or others). Therefore, the hypogonadism remained unexplained.

In 21.1 % of men with a complete azoospermia in the native ejaculate, there was a history of unilateral or bilateral undescended testicles with late operation. An incomplete, non-obstructive azoospermia was diagnosed in men (12.3 %) with normal-sized testicles, but where sperm cells could only occasionally be recovered following extensive search. In later testicular biopsies of these men, there were some small spots of full spermatogenesis. An obstructive azoospermia was only diagnosed in 1.8 % of all azoospermic men.

There was no case in which a CFTR mutation was detected. There was also no Klinefelter Syndrome and no Y-chromosome microdeletions found in the FMTS group.

Complete diagnostic workup with histopathological finding was also available for all 192 men in the FMES group (Fig. 2). These men were referred to our center after external testicular biopsy but with positive sperm detection in the ejaculate later. Cryptozoospermia due to unexplained hypogonadism with decreased size of the testicles and no further pathological findings was the most frequent diagnosis in the FMES group (45.4 %). Idiopathic cryptozoospermia with normal-sized testicles was the second rate diagnosis (30.7 %). Medical history analysis was negative for speculative causes of this idiopathic cryptozoospermia (state after genital or severe general infections, preterm labor, malignant disease or other concomitant diseases) and karyotypes were sound. In the FMES group though, we found autosomal translocations in 1.6 % indicating a genetic cause. Very importantly, in 3.7 % of all 192 testicular biopsies, a clinically unapparent malignant tumor or a precancerosis was detected. Interestingly, in the FMES, only 12.5 % of the men had a history of unilateral or

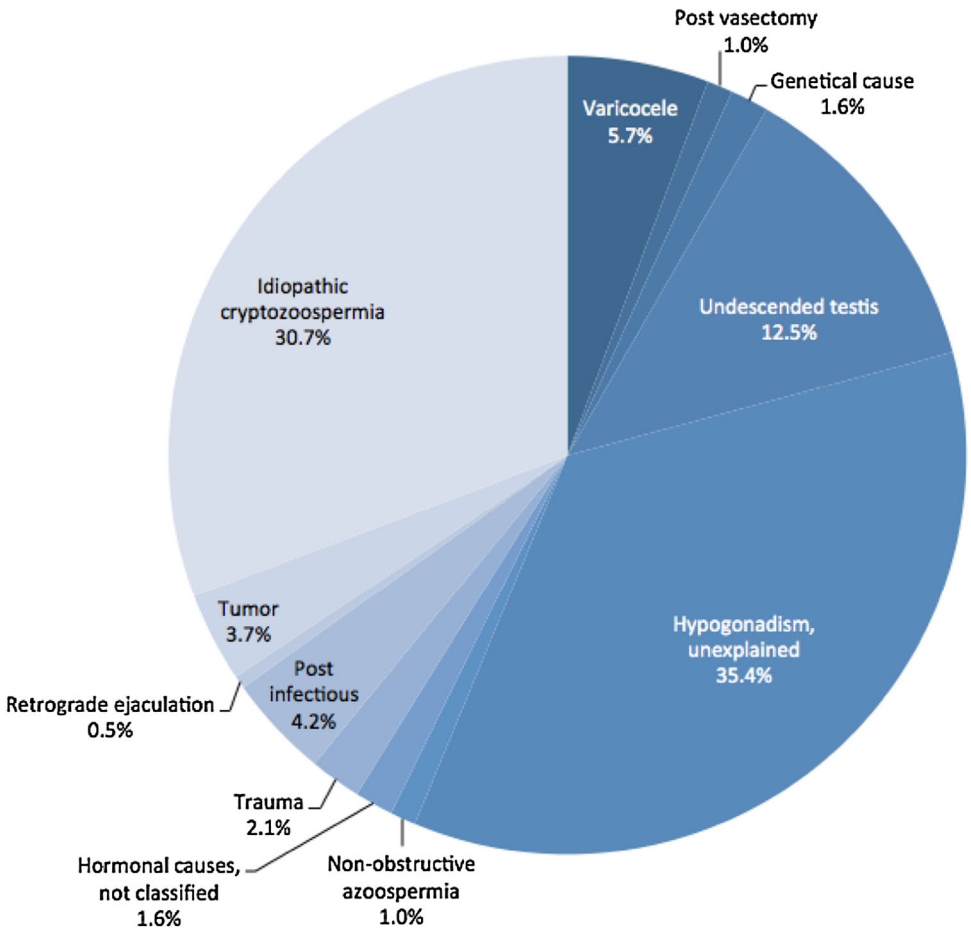

Fig. 2 Different etiologies of infertility in men with a cryptozoospermia (ejaculated sperm cell group, FMES)

bilateral undescended testicles compared to 21 % in the FMTS group.

Baseline characteristics and ICSI results for both study groups are shown in Table 1. In the FMTS group, 1.05 cycles per couple were performed and three couples underwent a maximum of two fresh cycles with repeated testicular biopsy. In the FMES group, 1.44 cycles per couple were performed and 4 couples underwent a maximum of four fresh cycles. Average age of the women, FSH in men, total number of oocytes, mean number of oocytes per cycle and mean number of mature oocytes per cycle were all not significantly different in both study groups.

A detailed comparison of the ICSI results (Table 1) in the FMTS group versus the FMES group showed no significant differences in fertilization rate, implantation rate, embryo development (only day three embryos, measured by the number of blastomers) and pregnancy rate. Stratifying all results for different female age classes again showed no significant differences. Seventy-six children were born altogether with a trend (i.e., not significant) towards a relatively higher number of children born in the FMES group (33 % of the couples finally successful versus 21 % in the FMTS group). In the FMES group, two couples had two live births and one couple had a live birth after the fourth fresh ICSI cycle. The follow-up calls (3–6 months after calculated day of delivery) did not reveal any malformation in all children born.

Discussion

There is a controversial debate on the impact of sperm source on ICSI outcome and, if both sources of sperm recovery are available, which source is preferable for microinjection [5–8, 21]. It has been postulated in the past that spermatozoa originating from the ejaculate of men with extremely reduced sperm cell concentration are damaged while transiting the male genital tract causing reduced fertilization and implantation rates [12–16, 22].

Very recently, preferential use of testicular spermatozoa was recommended for microinjection, especially when previous ICSI cycles with ejaculated spermatozoa failed [6]. However, testicular biopsy is an invasive surgical procedure with potentially irreversible testicular tissue damage, postsurgical complications and additional costs for patients and health insurances.

Table 1 Overview of the results of ICSI cycles according to the source of sperm cells in couples with severe male factor infertility

Results	FMTS group	FMES group	$P < 0.05$
N (couples)	57	192	
N (cycles)	60	277	
Cycles per couple (range)	1.1 (1–2)	1.4 (1–4)	Significant
Average age of the women	34.3 ± 7.3	33.5 ± 7.2	ns
FSH (men) mIU/ml	9.9 ± 7.5	11.4 ± 0.3	ns
Total number of oocytes	709	3,163	
Mean number of oocytes per cycle	11.7 ± 6.4	11.4 ± 6.0	ns
Mean number of mature oocytes per cycle	9.9 ± 6.2	9.4 ± 5.1	ns
Fertilization rate (%)	46.8	47.6	ns
Cycles with embryo transfer	54	254	
Total numbers of embryos transferred	105	526	ns
Embryo transfer rate (%)	90	92	ns
Embryos transferred per cycle	1.7 ± 0.8	1.8 ± 0.7	ns
Mean number of blastomers of the top embryo[a]	6.2 ± 2.6	6.4 ± 2.3	ns
Implantation rate (%)	18.5	18.6	ns
Total pregnancy rate per cycle, % (*n*)	21.7 (13)	30.6 (85)	ns
Total pregnancy rate per embryo transfer, % (*n*)	24.1 (13)	33.5 (85)	ns
Biochemical pregnancy rate per cycle, % (*n*)	0	2.5 (7)	
Biochemical pregnancy rate per ET, % (*n*)	0	3.7 (7)	
Clinical pregnancy rate per cycle, % (*n*)	21.7 (13)	22.0 (78)	ns
Clinical pregnancy rate per ET, % (*n*)	24.1 (13)	24.0 (78)	ns
Abortion rate per cycle, % (*n*)	3.3 (2)	6.8 (19)	ns
Abortion rate per ET, % (*n*)	3.7 (2)	7.5 (19)	ns
Ectopic pregnancy rate per cycle, % (*n*)	0	0.4 (1)	
Ongoing pregnancy rate per cycle, % (*n*)	18.3 (11)	20.9 (58)	ns
Ongoing pregnancy rate per ET, % (*n*)	20.4 (11)	22.8 (58)	ns
Life birth rate per embryo transfer, % (*n*)	20.4 (11)	22.8 (58)	ns
Total number of life births	11	58	ns
Total number of children born	12	64	
Singles	10	52	
Twins	2	12	

[a] Only day 3 embryos were taken into account

This study's data were extracted from a 10-year basic data set deriving from our center [23]. To our knowledge, it is one of the largest studies comparing differences in ICSI outcome using either testicular or ejaculated motile sperm cells.

Our results confirm previously reported findings that the injection of testicular or ejaculated spermatozoa results in similar fertilization and implantation rates [5]. Even in men with a high incidence of DNA damage, the microinjection of ejaculated or testicular spermatozoa in ICSI did not influence the fertilization rates [20].

Some studies report differences using either source of spermatozoa, higher rates of normal fertilization when using testicular spermatozoa [21, 24], whereas one case control study, very similar to our study here, found significantly lower fertilization rates using fresh testicular spermatozoa [10]. Ben-Ami et al. [6] report higher pregnancy and baby-take-home rates with the use of testicular spermatozoa although these cycles were performed after a series of cycles with ejaculated spermatozoa had failed in the same patient. This bias was partly overcome by transferring significantly more embryos in cycles with testicular spermatozoa.

It has been hypothesized that in case of a severe male infertility, the spermatozoa are much more sensitive to reactive oxygen species and a possibly cytotoxic milieu during the post-testicular passage [12, 22]. This cytotoxic milieu may increase the percentage of spermatozoa with DNA damage [20]. Our clinical results do not support this hypothesis of post-testicular damage during storage and passage of testicular spermatozoa through the epididymis.

Certainly, we are aware of some limitations of the present study. It is a retrospective cohort study with differing sample size in the groups. The classification of severe male infertility into different etiologies by histopathological and clinical findings is somewhat subjective of course [25, 26]. The higher rate of "basically" fertile men with previous vasectomy in the FMTS group might be discussed to cause a bias with a favorable impact on this group's results because of a better intrinsic sperm quality. However, this is not the case because we do not find any significant differences in the outcome of ICSI between both groups. If we had found any differences in favor of FMTS, a remove of cases post-vasectomy would have been necessary.

Our analysis of two groups of different etiologic composition underlines that etiology of severe male infertility has very limited clinical impact, as long as fresh, motile and morphologically normal spermatozoa can be used for microinjection. These spermatozoa obviously have similar intrinsic quality, which confirms results by French et al. [4].

From our results, we conclude that the source of spermatozoa has no impact on the results of ICSI cycles. Therefore, in case of cryptozoospermia, we recommend to preferentially use ejaculated spermatozoa to prevent men from testicular biopsy implying unnecessary risks and costs. A switch to testicular spermatozoa in patients with a cryptozoospermia should be performed though, if four or more cycles with ejaculated spermatozoa fail [6, 24, 27].

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Conflict of interest The corresponding author and all co-authors have nothing to disclose. All the materials contained in the manuscript have not been published and are not being submitted elsewhere for publication. All authors agree to submission of the manuscript to Archives of Gynecology and Obstetrics.

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