

Fertility biomarkers predict differential survival in a long-term follow up study of male infertility. Capturing and treating heterogeneity

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1. Abstract:

We found that low sperm counts and other impaired biomarkers predict differential in survival among otherwise healthy subjects in a long-term fertility follow-up. Here we report on possible hidden effects of the heterogeneous composition of the sample. Thus, the differences in lifespan for men with lower sperm concentration might be an artefact because of presence for unobserved heterogeneity. A convenient way to demonstrate the intensity of heterogeneity on the population hazard could be done in use of Frailty Models regarding the individual frailty. Heterogeneity can be indicated as the variance in frailty. If $\sigma^2 = 0$ indicates no heterogeneity while with increasing value of σ^2 the effect of heterogeneity become more relevant and leads in extreme cases to an unimodal shape of the hazard function. The final intention of our research project is the estimation of the hazard function for fertile and subfertile men under consideration of diversity in age-structure and some hidden effects of co-morbidity regarding the diseases of the genitourinary.

2. Introduction

The validity of epidemiological studies have to be mistrusted in case if the hidden effects of composition in subgroups for example differences in age-structure or individual risks will not be considered in many analysis. For some cases the estimation or the comparison of individual hazard rates or population intensities become more weak if the subgroups are too heterogeneous. The modelling of population intensities or hazard rates may often be effected by two mayor reasons.

1. The individual hazard changes over time and during cohorts.
2. The self-selection of higher-risks individuals leads to an earlier withdrawal in the risk population. The high-risk population will eventually turn to a low-risk population.

Most of all previous studies in context of the association of life span and fertility status showed any significant differences in mortality between fertile and subfertile men.

In a thesis (Groos, 2006) about the lifespan of men with fertile and subfertile fertility status indicates one 1.43 higher risk to fail for subfertile men according to men with normal sperm counts. The lower lifespan for men with a lower sperm counts could be an artefact because of the heterogeneous composition of the two subgroups. If the individual risk is still much higher in the indicated higher risk subgroup from beginning at time of observation the selection of the so called “bad” risks initially can be proceed much faster and may leads to the Mortality Cross over one of the paradoxical effects for heterogeneity. Multiplicative effects (in genetics or health) of risk factors could be answerable for this phenomenon of the Mortality Cross over but their impact diminished with ages. Rothman, Greenland (1998) show in a study of British doctors the influence of smoking on coronary death declines with ages.

In this paper we want to prove the effect of heterogeneity in order to defective survival curves. The intention of our research isn't only focused on testing the effect of heterogeneity more over we want to implicate indicators for evaluating the intensities. A convenient way to demonstrate the intensity of heterogeneity on the population hazards could be done by using Frailty Models. The advantages of the models are not focusing only on the estimations for the regression between the hazards and the covariates more over to capture diversities on the individual hazards (the frailty). More precisely the variance in individual frailty can be account for some misleading or controversial results for instance by the comparison the estimations of the Proportional Hazard Models with Parametric Models like the Frailty Gamma Models. The

Frailty Model not only takes for granted the existence of heterogeneity it can also measure the intensities of heterogeneity. If the individual frailty variance $\sigma^2 = 0$ indicates no heterogeneity while with increasing value of σ^2 the effect of heterogeneity becomes more relevant and leads to unimodal shape of the hazard function resulting in feasible wrong interpretations for the estimated parameters. More over some conclusions of our findings could lead to some potential recommendation to preserve some restriction of the models in allowing more covariates like age-specific or chronic diseases affecting the testicular malposition or some genetic reasons accounting for differences in individual frailty in infertility and survival.

3. Methods

In most of the studies analysing survival in populations, typically only a few covariates like age or sex are known. But there are more other variables also influence on survival for example health status, life style, smoking, occupation and genetic risk factors. But it is nearly always impossible to include all important risk factors perhaps because we have little or no information on the individual level. The relevance of these risks factors or even that the factors exist is also often unknown. For successive data analysis we have to consider two different sources of variability in duration data: first the theoretically predictable variability for the risk factors being observed and second the fact of heterogeneity which is theoretically unpredictable.

Hougaard (1991) justifies a separate analysis for hidden heterogeneity because these unobserved effects could lead to some unexpected results or to alternative interpretations for decreasing or non-proportional hazards. In special cases the individuals with higher risk tend to more frail and die much faster and turn to a more or less selected group with lower risks. The estimation for the individual hazards without considering the unobserved frailty could be resulting in an overestimation for the hazard function. Treating these selection effects could be done by a Mixture(gamma) Frailty Model. The assumption follows by a mixture of individuals with different risks and the hidden effects are defined by a mixture variable called the frailty. The frailty is a random variable implicating different statistical distributions like binary, gamma and log-normal showing the qualitative and quantitative differences. The variance of the frailty in populations determines the degree of heterogeneity in the study population.

The so called “basic” Frailty Model based on the assumption of the proportional hazard models being affected conditional on the random effect (frailty). The individual hazard only de-

depends on an unobservable, age-independent random variable Z , which acts multiplicatively on the baseline function μ_0 .

$$\bar{\mu}(x) = Z\mu_0(x)$$

Z is a random mixture variable, varying across the population. The scale factor (function for age of death) is common to all individuals with a given equal baseline hazard function μ_0 and a frailty distribution standardized to $EZ = 1$. The variance parameter $\sigma^2 = V(Z)$ indicates the intensities of heterogeneity across the population in baseline risk. If the value of σ^2 is small, then Z converge to 1. But when σ^2 is increasing Z become more loose and the individual hazard μ_0 has been higher effected by heterogeneity. As general assumption Z is a gamma-distributed random variable ($Z \sim G(k, \lambda)$) with mean 1 and variance σ^2 , then $k = \lambda$, and

$\sigma^2 = 1/\lambda$. Then $H(x) = \int_0^x \mu_0(u)du$ is defined as cumulative baseline hazard, the observed mortality $\bar{\mu}(x)$ can be written (see also the notation by Vaupel et al. 1979) as:

$$\bar{\mu}(x) = \frac{\mu_0(x)}{1 + \sigma^2 H(x)}$$

The marginal survival function $S(x)$, can be presented as:

$$S(x) = \left(1 + \frac{1}{\lambda} H(x)\right)^{-k} = \left(1 + \sigma^2 H(x)\right)^{-(1/\sigma^2)}$$

for homogenous populations $\bar{\mu}(x) = \mu_0(x)$, with variance $\sigma^2 = 0$ and individual frailty = 1.

Then one follows the L'Hôpital rule with $S(x) \rightarrow \exp(-H(x)) = S_0(x)$, (see also Abramowitz and Stegun 1972) to demonstrate the correlation between the death rate and survival function. Only for the homogenous cases the assumption of the proportional characteristics from death rate and lifespan will not be violated, because the reduction in death rates on one level will be resulting in an extension of the life expectation on the same level. The risk of dying is corresponding for all individuals by the given age. In fact, the population characteristics like hazard, survival function and the average lifespan are valid for all members of the population, when the cumulated parameters for the hazards etc. could be construed as the mean factors for the surviving individuals. In most of all epidemiological studies the population for different subgroups don't tend to be homogenous. For these cases the simple assumption for the population hazard being the average hazard among for the individuals have to be relativized.

Vaupel et al. (1979) suggested a gamma distribution for the variation in values for the frailties in the study population. The use of a gamma distribution for the frailty variation has two essential advantages: First the frailty distribution of the survivors at any given age follows a gamma distribution specified with the same shape parameter but a different scale parameter, second the frailty distribution among persons dying at any age is also gamma distributed with an increasing shape parameter by one and a scale parameter, as a function for the age of death. The gamma distribution is one of the most flexible statistical distributions and can be used alternatively for any other distributions.

But it has to be mentioned that there aren't any biological reasons existing to specify the motivation in use for the gamma distribution. The unexplained heterogeneity (frailty) can be unshared or shared among individuals. If individuals are sharing the frailty it is common to a several group of individuals leading to slight different estimations for regression coefficients, but the decisive differences can be detected for the Cox Model and the Frailty Model.

The following example will show the variation in estimation for the covariates the Proportional Hazard Model in comparison to the Frailty Gamma Model. We just refer to a study of McGilchrist and Aisbett (1991) who have studied the time to infection in kidney catheters for dialysis patients. The 82 patients enrolled in the study adapt one or more (highest to 8) observation periods. A subset of the data includes the first two observation periods of 38 patients. For simplification the analysis was restricted in using only the two covariates age and sex and the two observation periods as independent variables. The analysis for the fitness of a Cox Model for the data was first presented by Hougaard (2000).

Table 1

	Proportional hazard Cox	unshared gamma frailty
age	0.0022 (0.0092)	0.0067 (0.0124)
gender	-0.0820 (0.3000)	-1.770 (0.0610)
σ^2		1.510 (0.980)

Estimates for log relative hazards and log likelihoods for various shared frailty models, for catheter infection data (Hougaard 2000)

Table 1 shows the not corresponding results for the estimated values of the covariates for the Cox Model and the Frailty Model. The estimates for the Cox Model seem to be biased downwards when the effects of unobserved heterogeneity will not be captured. The Frailty Gamma Model is able to treat the hidden heterogeneity but the high value for the standard deviation doesn't clarify the existence of no unobserved heterogeneity. When $\sigma^2 = 0$ is valid for the Frailty Gamma Model, then it can be reduced to the standard Cox Proportional Hazards

Model (a semi-parametric approach). Because of its unspecified baseline hazard the intention of the Proportional Hazard Model is only focusing on the estimation of the regression directly on the population hazard and the covariates. On the one hand the estimation procedures become more simplified without the need to frame any assumption for the individual hazard. But on the other hand the specification of the hazard function in the Cox Model only depends on the impact of parametric modelling for the covariate-vector (partial-likelihood). In using a partial-likelihood-approach the shapes of the function neither nor the full intensities of the population hazard could be identified.

With following analysis we want to prove if there are any hidden effects of heterogeneity can be detected for our data set. Although the frame of our work is to retain which approach the Frailty Gamma Model or the standard Cox Model is reliable for our regression estimates and the specification of the hazard function.

4. Data set

The data set includes all infertility patients who had attended the fertility and sterility office of the department of andrology at Marburg University Hospital for semen analysis between 1949 and 1998 who were born before 1942. Until now we have analyzed more than 2.000 medical records. After excluding cases for diseases which were identified that might be effect fertility, we have to distinguish between two subgroups. The first will include fertile and the second subfertile men. The assignment to both groups was carried out by the analysis of semen samples on the basis of medical records by sperm concentration which allows according to the WHO declaration a classification to infertile by a sperm concentration of less than 20 Mio. per mL and to fertile men by ≥ 20 Mio. per mL. Otherwise it is useful to divided the subfertile cases into azoospermics with none sperms in ejaculate and into oligospermics with less than 20 Mio. but more than zero sperms in ejaculate because we would expect differences in survival within the subfertile subgroup. For our actual analysis we include patients who have died until that date so that the youngest were at an age of 65 years. Excluded are men of other ethnicities because we cannot rule out differences in health status and living conditions between people with foreign nationalities.

Table 2 Classification for sperm concentration WHO (1999)

sperm concentration	fertility status	
≥ 20 Mio./mL	fertile	normosperm
< 20 Mio./mL	subfertile	oligosperm
0 Mio./mL		azoosperm

Our data set contains 2297 cases thereof 890 will be lost to follow up because it was not possible to identify the status of vitality for these cases. Therefore our estimations for the survival only refer to 1407 males with fertile or infertile status. Suddenly we would not expect a higher lost to follow up quota for the azoospermic cases because patients might be exposed in higher risks of physical constrictions staying longer under medical surveillance. Not very surprising are the higher intensities for azoospermic males in row death rates because of the fact of an decreasing amounts in sperms is related with maturing ages followed by higher mortality. (According to the mean age of first examination one can't specify any difference into the three subgroups.)

Table 3 Descriptives for azoosperm, oligosperm and normosperm patients

status of vitality	azoosperm	oligosperm	normosperm	Missing	total
alive	129	195	647	2	973
dead	78	91	264	1	434
total	207	286	911	3	1407

fertility status	lost to follow up	row death rates	mean age at first examination
azoosperm	0,4202	0,3768	34,44
oligosperm	0,3889	0,3182	34,46
normosperm	0,3777	0,2898	34,75

5. Results

5.1 Nonparametric estimates

For both subgroups one can detect an increase for the failure rates at 20 years after the day of first examination. Subfertile cases (see Chart 1) purpose to be less fail during 20 and 40 then the hazards, because their curve runs slightly under the estimated curves for the fertile ones. In comparison for the three subgroups (azoospermic, oligospermic and normospermic) more surprise is founded of the runs for the hazards of normospermic and oligospermic (see Chart

2) cases are more similar to each other more than for the might expected in equality for oligospermic and azoospermic cases. Referring to the results in Table 3 one can't conclude any significant differences in hazards according fertility status.

Table 3 Test for equality of survivor function

	Log-rank	Tarone-Ware	Wilcoxon	Peto
subfertile-fertile	2.7 (0.1002)	2.41 (0.1206)	1.40 (0.2367)	1.13 (0.2884)
azoo-oligo-normospermic	7.34 (0.0254)	6.34 (0.0420)	4.30 (0.1164)	3.83 (0.1475)

Regarding age-specific effects in the regression models will lead to more reliable results for the estimations precisely to analyse differences in failure for fertile and subfertile males. Because of the fact that the sperm counts is decreasing with matured age one possibly can detect higher hazards for any subgroup. Also the impact of the age-specific effects on the survival can be more intensive then concealing the “real” effects of different fertility status predicting the difference in survival. More over our data set includes the information of male fertility status for different cohorts with the oldest individual born in 1892 and the youngest ones born in 1942. The observed variation in cohorts could be also responsible for differences in survival then the older may be higher exposed to fail in comparison to the youngest ones. Otherwise the sensitivity on exposure for censoring into the subfertile subgroup followed by one more intensive pre-selection in azoospermic men. Because of the supposed higher pre-selection the high-risk cases may be earlier drop out from the study-population then more cases with lower susceptibility remain.

5.2. Results from Cox Proportional Hazards Models estimates

The estimation results of the Cox Models clarify the closely interactions between age-specific effects and fertility status to corresponding with risks to failure. It is also shown that the varieties on survival aren't really significant by comparing fertile and subfertile cases. Nevertheless the fertile cases face 3.5% higher risk (Table 4) in failure time than men with sperm counts less than 20Mio/mL. Awaiting the within-group correlation for cohorts (Table 5), fertile men will be slightly less exposed to fail than subfertile ones but the estimations are not significant. The findings for three subgroup comparison indicate 16% and 22% higher hazard (Table 6) for normospermic and oligospermic men referred to the hazards of the azoospermic ones. The intensities of stringent exposure for normospermic and oligospermic males decline to 2.3% and 9.7% (Table 7) with attending the within-group correlation for the cohorts.

Because of its simplification in only estimating the regression between the population hazard and the covariates without regarding the within-group variance on the individual hazard one can't rule out the impact of unobserved heterogeneity for the analysis. It has to be mentioned that effects heterogeneity on the individual hazard can lead to biased estimation results if it will not be detected. Another reason to scrutinize the estimations of the Cox Model is justify with its assumption of the semiparametric model (Ghosh and Ghosal, 2006) which makes the interpretations of the regression coefficients more complicated. Hence we used full-parametric Frailty Models to figure out the existence and the intensity of unobserved heterogeneity for our study population.

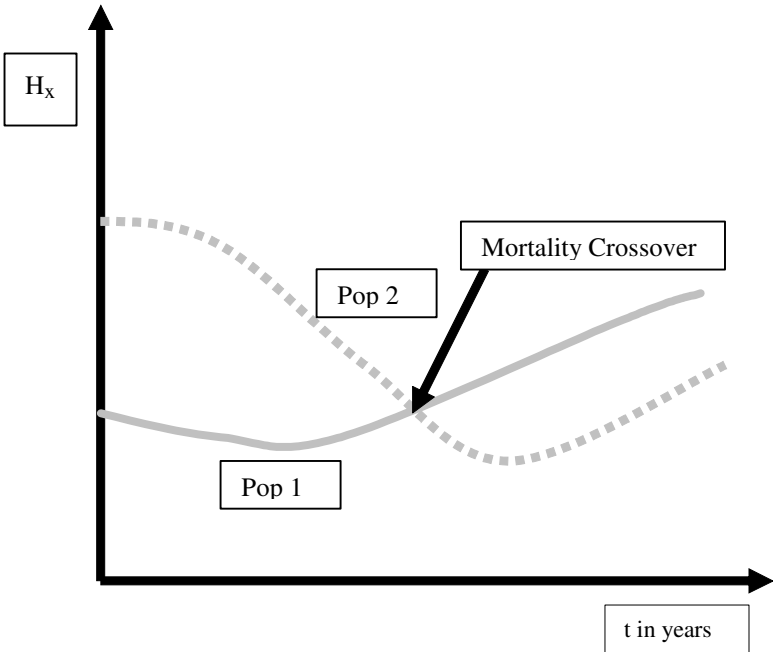
5.3. Results from Frailty Model estimates

Only the models with the Gompertz distributed baseline functions provide consistent results for our frailty estimations. For both models (the comparison for fertile and subfertile and the subdivision in azoospermic, oligospermic and normospermic males) one can determine the evidence for unobserved heterogeneity although the values for (θ the variance on the individual frailty) with 0.257 and 0.261 are very low, but the likelihood-ratio test for $H_0: \theta = 0$ would be rejected, here at the 0.000 level. Consequently we have to mistrust the estimations from the Cox Proportional Hazards Models, because these findings are only unbiased in case of $\theta = 0$ with an individual frailty = 1. For our special cases we can determine in the Frailty Gamma Model for comparing among the two subgroups (fertile and subfertile) one slightly lower risk (8%) to fail for fertile men, conditionally be effected by the variance of individual frailty. Furthermore for three subgroup analysis the findings of the Frailty Gamma Model show higher hazard (23%) for oligospermic men referring to the hazards of azoospermic ones. Finally one can conclude only significant differences in failure time for oligospermic and azoospermic men. Possible causes for this disparity could be explained by age-specific effects in decomposition and the different degrees on sensibility of susceptibility according Censoring.

6. Discussion

In many epidemiological studies the effects of unobserved heterogeneity have to be considered otherwise the estimation results will be biased. It is also shown that the findings for semiparametric and full-parametric models can be differing. Semiparametric models like the Cox Proportional Hazard Model assume constant hazard that means all individuals have the equal hazard risk. This assumption might not be really realistic because the individual hazard can also vary in course of the process time particularly if the within-group consistence of age also changes over time. In extreme cases the proportional-hazard-condition is strictly violated and leading to Mortality Crossing over then the consistence of the study population is totally different at the end than to the population at the time of the beginning for instance then the individuals turn from the high risk to the low risk population. In contrast Frailty Models allowing a mixture on the individual hazard level and purvey more reliable estimation results irrespective if the proportional-hazard-condition will be violated or not.

Chart 3 Mortality Crossover (extreme case)



In our analysis the intensity of unobserved heterogeneity is very low but therefore one have to consider for the estimations. It has to be mention that age-specific effects and within-group correlation in cohorts can be assert as the major reason for the disparity for azoospermic and oligospermic men and might be better predictors for the population hazards. For any other prospective covariates like preliminary diseases, genetics or any other health specific indica-

tors one can't find any association to the hazard function. We have to admit that our data set includes only minor information about co-morbidity on the individual level, because the examining doctors often missed to note all relevant preliminary diseases regarding the system of genitourinary. So far we are only focusing on the analysis of the none-censored cases, because we could not identify the exact failure for all cases. Censoring also can evoke an untimely preselection in the azoospermic subgroup effecting that the high risk cases will be more selected and the azoospermic tend to less fail.

Major causes of male infertility

Eventually we are presenting a short overview about the causes for male infertility. About 15% of couples being asses to infertility, nearly 40% of the time the infertility are due to male factors. More of the half male infertility cases could be related to defined reason like varicocele, infections, hormone imbalances, blockage of the reproductive tract ducts, previous surgery, x-ray and some behaviour specific causes lime consumption in tobacco or drugs and the exposure of heat for instance frequent attendance in sauna or tanning booth. Table 13 presents the frequency of these different reasons resulting in male infertility.

Table 13 Major Causes of male infertility

idiopatic subfertility	1,7%
varicocele	16,6%
infections	9,0%
hypogonadism	8,9%
abnormity of the testes	8,5%
disruption in deposition of semen	5,8%
chronical diseases	5,0%
immune factors	4,2%
testes tumor	2,3%
obstructions	1,5%
others	6,6%

(Dorn 2007)

In spite of the status of infertility for some male patients could not be explained by these present known factors. That's why the genetic disruptions become one of the most commonly diagnosed reasons for male infertility. In this context the Klinefelt-Syndrom with 5 percent is the most frequent diagnosed genetic disruption for infertile men with 44 times higher prevalence in comparison to the fertile ones (Küpker et. al 2007). The classical KS is caused by the

meiotic non-disjunction of the X-chromosome. In phenotypes these men have low volumes of testes, being more affected of azoospermia and having low concentrations in testosterone. The prevalence of chromosomal abnormality in infertile men varies from 2,1 – 8,6 percent. The major chromosomal disruption of men with defections in spermiogenesis is followed by the 47,XXY-Karyotype, with 90 % of all male patients. During the last decades many studies have been focused on searching the association of the abnormalities on Y-Chromosome regarding the existence on male sub- or infertility. The responsible site of the spermiogenesis has been located on the long arm of Y-Chromosome (Yq11) and the two genes YRRM₁ and YRRM₂ causing the intensities of azoospermia.

In a study of genetics in male infertility Turek (2008) concludes that with diagnosis of oligospermia the risk of chromosomal abnormalities is 2% and the risk of Y chromosome microdeletions of Y chromosome is 6-8% higher in comparison to the general population. In general, the lower the sperm count, the higher the change that a genetic cause is present. The Y chromosome microdeletion is often been inherited to the offspring if the father have an imbalance in chromosome material. Then it's often assumed that any son having inherited a defection on Y chromosome will also be infertile like his father. But so far, there are known only a few observed cases of son being born to fathers with Y chromosome deletions after conception by assisted reproduction.

Table 14 Quick Reference for Genetic Risks associated with Common Male infertility Diagnoses.

Diagnosis	Chromosome Abnormality Risk	Y chromosome Microdeletion Risk	CF Mutation/5T Allele Risk
Oligospermia (< 5 million sperm/mL)	2-7%	6-8%	Same as general popul.
Nonobstructive Azoospermia	15%	13%	Same as general popul.

Turek (2008)

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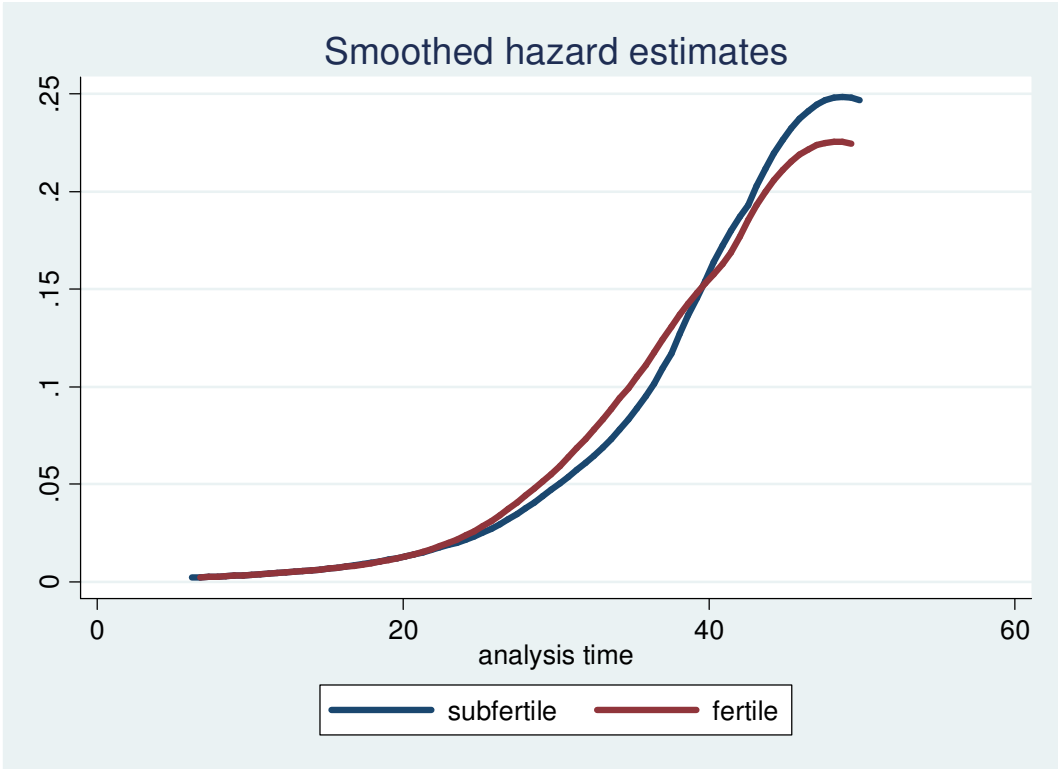
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Tables

Times	Event	Age	Sex	Type	Times	Event	Age	Sex	Type
8, 16	1, 1	28	1	3	23, 13	1, 0	48	2	0
22, 28	1, 1	32	1	3	447, 318	1, 1	31, 32	2	3
30, 12	1, 1	10	1	3	24, 245	1, 1	16, 17	2	3
7, 9	1, 1	51	1	0	511, 30	1, 1	55, 56	2	0
53, 196	1, 1	69	2	1	15, 154	1, 1	51, 52	1	0
7, 333	1, 1	44	2	1	141, 8	1, 0	34	2	3
96, 38	1, 1	35	2	1	149, 70	0, 0	42	2	1
536, 25	1, 0	17	2	3	17, 4	1, 0	60	1	1
185, 177	1, 1	60	2	3	292, 114	1, 1	43, 44	2	3
22, 159	0, 0	53	2	0	15, 108	1, 0	44	2	3
152, 562	1, 1	46, 47	1	2	402, 24	1, 0	30	2	3
13, 66	1, 1	62, 63	2	1	39, 46	1, 0	42, 43	2	1
12, 40	1, 1	43	1	1	113, 201	0, 1	57, 58	2	1
132, 156	1, 1	10	2	0	34, 30	1, 1	52	2	1
2, 25	1, 1	53	1	0	130, 26	1, 1	54	2	0
27, 58	1, 1	56	2	1	5, 43	0, 1	50, 51	2	1
152, 30	1, 1	57	2	2	190, 5	1, 0	44, 45	2	0
119, 8	1, 1	22	2	3	54, 16	0, 0	42	2	3
6, 78	0, 1	52	2	2	63, 8	1, 0	60	1	2

The sex is given as 1 for males and 2 for females. The type of disease is specified with 0 = glomerulonephritis, 1= acute tubular nephropathy, 2 = polycystic kidney disease and 3 = others.

Graph 1 Smoothed hazards for subfertile and fertile men



Graph 2 Smoothed hazards for azoospermic, oligospermic and normospermic men

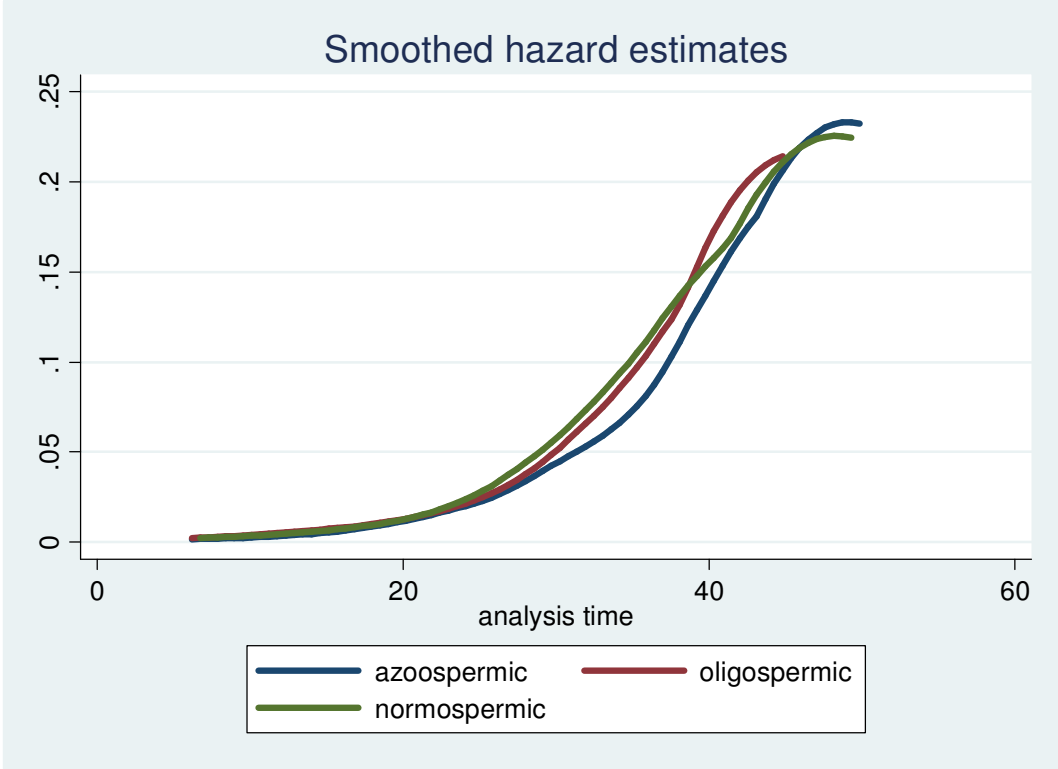


Table 4 Cox Model for subfertile (reference) and fertile men with age at first examination, tested by the shared frailty for cohort (gamma-distributed)

Cox regression -- Breslow method for ties

```
No. of subjects =          1398          Number of obs  =          1398
No. of failures =          1398
Time at risk    =          49483
Log likelihood  = -8593.6908          LR chi2(2)      =          450.83
                                                Prob > chi2    =          0.0000
```

_t	Haz. Ratio	Std. Err.	z	P> z	[95% Conf. Interval]	
fertile	1.035488	.0583243	0.62	0.536	.927258	1.15635
age_exam	1.10146	.0046131	23.07	0.000	1.092456	1.110539

Table 5 Cox Model for subfertile (reference) and fertile men with age at first examination, tested by the shared frailty for cohort (gamma-distributed)

Cox regression --

```
Breslow method for ties          Number of obs  =          1398
Gamma shared frailty           Number of groups =           2
Group variable: Cohort
```

```
No. of subjects =          1398          Obs per group: min =          659
No. of failures =          1398                        avg  =          699
Time at risk    =          49483                        max  =          739
```

```
Log likelihood  = -8247.5141          wald chi2(2)    =          1123.04
                                                Prob > chi2    =          0.0000
```

_t	Haz. Ratio	Std. Err.	z	P> z	[95% Conf. Interval]	
fertile	.9699175	.0547625	-0.54	0.589	.8683107	1.083414
age_exam	1.183577	.0059541	33.50	0.000	1.171965	1.195305
theta	.7471742	.6759626				

Likelihood-ratio test of theta=0: $\chi^2(01) = 692.35$ Prob>=chibar2 = **0.000**

Table 6 Cox Model for subfertile (reference) and fertile men with age at first examination, and Cohort

```

failure _d: 1 (meaning all fail)
analysis time _t: duration_observed

Iteration 0: log likelihood = -8819.1034
Iteration 1: log likelihood = -8277.9192
Iteration 2: log likelihood = -8239.4763
Iteration 3: log likelihood = -8239.321
Iteration 4: log likelihood = -8239.321
Refining estimates:
Iteration 0: log likelihood = -8239.321

Cox regression -- Breslow method for ties

No. of subjects =      1398           Number of obs =      1398
No. of failures =      1398
Time at risk    =      49483

Log likelihood   =     -8239.321      LR chi2(3)         =     1159.56
                                           Prob > chi2        =       0.0000

```

_t	Haz. Ratio	Std. Err.	z	P> z	[95% Conf. Interval]	
fertile	.9712316	.0548295	-0.52	0.605	.8694998	1.084866
age_exam	1.184067	.0059682	33.52	0.000	1.172427	1.195822
cohort	7.101895	.5568679	25.00	0.000	6.090186	8.28167

Table 7 Cox Model for azoospermic (reference), oligospermic and normospermic men with age at first examination

```

Cox regression -- Breslow method for ties

No. of subjects =      1398           Number of obs =      1398
No. of failures =      1398
Time at risk    =      49483

Log likelihood   =     -8591.2883      LR chi2(3)         =     455.63
                                           Prob > chi2        =       0.0000

```

_t	Haz. Ratio	Std. Err.	z	P> z	[95% Conf. Interval]	
normo	1.158824	.0902608	1.89	0.058	.994758	1.34995
oligo	1.222269	.1124401	2.18	0.029	1.020615	1.463764
age_exam	1.101393	.0046042	23.10	0.000	1.092406	1.110454

Table 8 Cox Model for azoospermic (the reference), oligospermic and normospermic men with age at first examination, shared frailty for cohort (gamma-distributed)

```

Cox regression --
  Breslow method for ties          Number of obs    =    1398
  Gamma shared frailty            Number of groups  =     2
Group variable: Cohort

No. of subjects =    1398          Obs per group: min =    659
No. of failures =    1398          avg =    699
Time at risk    =   49483          max =    739

Log likelihood =  -8247.0023        wald chi2(3)     =   1127.29
                                          Prob > chi2      =    0.0000

```

_t	Haz. Ratio	Std. Err.	z	P> z	[95% Conf. Interval]	
normo	1.02353	.0801786	0.30	0.767	.8778524	1.193383
oligo	1.097704	.1013737	1.01	0.313	.9159589	1.31551
age_exam	1.18325	.005952	33.45	0.000	1.171641	1.194973
theta	.7445728	.6738064				

Likelihood-ratio test of theta=0: chibar2(01) = 688.57 Prob>=chibar2 = **0.000**

Table 9 Cox Model for azoospermic (reference), oligospermic and normospermic men with age at first examination, shared frailty for diseases (gamma-distributed)

```

Cox regression --
  Breslow method for ties          Number of obs    =    1398
  Gamma shared frailty            Number of groups  =     3
Group variable: diseases

No. of subjects =    1398          Obs per group: min =    39
No. of failures =    1398          avg =    466
Time at risk    =   49483          max =   1245

Log likelihood =  -8586.995        wald chi2(3)     =   543.56
                                          Prob > chi2      =    0.0000

```

_t	Haz. Ratio	Std. Err.	z	P> z	[95% Conf. Interval]	
normo	1.142602	.0895968	1.70	0.089	.9798241	1.332421
oligo	1.207337	.111724	2.04	0.042	1.007071	1.447426
age_exam	1.10233	.0046368	23.16	0.000	1.09328	1.111456
theta	.0896413	.0894836				

Likelihood-ratio test of theta=0: chibar2(01) = 8.59 Prob>=chibar2 = **0.002**

Table 10 Cox Model for azoospermic (reference), oligospermic and normospermic men with age at first examination and Cohort

```

failure _d: 1 (meaning all fail)
analysis time _t: duration_observed

Iteration 0: log likelihood = -8819.1034
Iteration 1: log likelihood = -8278.1672
Iteration 2: log likelihood = -8239.0095
Iteration 3: log likelihood = -8238.8402
Iteration 4: log likelihood = -8238.8402
Refining estimates:
Iteration 0: log likelihood = -8238.8402

Cox regression -- Breslow method for ties

No. of subjects = 1398           Number of obs = 1398
No. of failures = 1398
Time at risk = 49483

Log likelihood = -8238.8402     LR chi2(4) = 1160.53
                                Prob > chi2 = 0.0000

```

_t	Haz. Ratio	Std. Err.	z	P> z	[95% Conf. Interval]	
normo	1.023256	.0801537	0.29	0.769	.8776231	1.193056
oligo	1.094572	.1010949	0.98	0.328	.9133286	1.311781
age_exam	1.183728	.005967	33.46	0.000	1.17209	1.195481
Cohort	7.072603	.5550579	24.93	0.000	6.064249	8.248626

Table 11 Gamma Frailty Model with Gompertz distributed baseline function for fertile and subfertile man

```

Gompertz regression -- log relative-hazard form
Gamma frailty

No. of subjects = 1398           Number of obs = 1398
No. of failures = 1398
Time at risk = 49483

Log likelihood = 607.16341     LR chi2(3) = 1309.62
                                Prob > chi2 = 0.0000

```

_t	Haz. Ratio	Std. Err.	z	P> z	[95% Conf. Interval]	
fertile	.9287006	.0637034	-1.08	0.281	.8118733	1.062339
age_exam	1.262607	.0138432	21.27	0.000	1.235764	1.290033
Cohort	9.647541	.8886556	24.61	0.000	8.053981	11.5564
/gamma	.2606552	.0089276	29.20	0.000	.2431574	.278153
/ln_the	-1.356648	.1852636	-7.32	0.000	-1.719758	-.9935379
theta	.2575226	.0477096			.1791095	.3702644

Likelihood-ratio test of theta=0: $\chi^2(01) = 42.85$ Prob>= $\chi^2 = 0.000$

