Journal of Pediatric Surgery 56 (2021) 360–367

Contents lists available at ScienceDirect

Journal of Pediatric Surgery

journal homepage: www.elsevier.com/locate/jpedsurg

Does open ovarian biopsy in prepubertal age affect ovarian reserve in a rat model?

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ARTICLE INFO

Article history:
Received 7 March 2020
Received in revised form 29 May 2020
Accepted 30 May 2020

Key words:
ovarian reserve
biopsy
child
animal model
fertility

ABSTRACT

Background: Partial resection of the ovary carries a possible risk of fertility reduction. We studied the influence of open ovarian biopsy on ovarian reserve, including anti-Müllerian hormone and follicle-stimulating hormone serum level evaluation, in a prepubertal rat model.
Methods: Interventions – the initial surgery was unilateral ovarian biopsy (38 rats, group B1, B2) or unilateral ovarian biopsy and ovarian resection of the contralateral gonad (38 rats, group BR1, BR2). The second operation was bilateral ovarian resection and total resection of the remaining ovary. All rats had hormone serum levels evaluated. The control group had only a blood test taken and bilateral ovarian resection done at the second intervention (30 rats, group C1, C2). The collected tissue was examined estimating follicle count and anti-Müllerian hormone immunoexpression.
Results: Anti-Müllerian hormone levels were significantly lower at the second intervention in the group BR2 but significantly higher in the group C2. Follicle-stimulating hormone levels were significantly higher in all but one group (BR2).
Conclusions: Biopsy itself might not reduce ovarian reserve if done properly but we should know its possible negative effects in the case of a single remaining ovary.

Our knowledge about damage to ovaries caused by various therapies is limited. Ovarian biopsy in the case of suspicion of a malignant tumor present in one of the gonads is an important method to diagnose the disease in the other ovary. The effect of this iatrogenic ovarian injury is still unknown. The management in the case of ovarian biopsy is based mostly on experience and beliefs of a surgeon and there are no guidelines regulating its use in children. To our knowledge, there is no research concerning ovarian function after biopsy in children or data on the risk of infertility as a result of this procedure (a search of PubMed: English language; 1969–2019; search terms: "ovarian biopsy" and "child").

Another procedure, based on partial resection of ovarian cortex, carrying a possible risk of fertility loss or its reduction, is the cryopreservation of ovarian tissue. Considering the growing number of patients recovering from cancer disease, the need to protect their fertility is imperative and becomes our duty. For instance, in a recent study by Furi et al., 21.2% of adolescent and young adult cancer patients in Japan suffered infertility due to chemo- or radiotherapy and gave up childbearing. Ovarian failure, one of the causes of infertility, can also be induced by genetic disorders, autoimmune pathologies, inflammatory or intrinsic disorders or by other surgical treatments involving the ovaries. Ovarian tissue cryopreservation is the only available option of fertility protection suitable for younger prepubertal patients [1–6]. Nevertheless, the available studies concern mostly the effects of cytotoxic therapy (researches in animal model and in humans; a search of PubMed: English language; 1969–2019; search terms: "ovarian cryopreservation" and "child").

The aim of this study was to determine the influence of open ovarian biopsy on the ovarian reserve in a prepubertal rat model.

Specific study aims:

1) assessing the influence of the unilateral ovarian biopsy on the ovarian reserve
2) assessing the influence of the unilateral ovarian biopsy on the ovarian reserve of this ovary in case of the contralateral ovary resection
3) assessing the correlation between the ovarian reserve and the time from the surgery

The presented study design was based on the following assumptions:

The main study hypothesis was that the influence of ovarian biopsy (in prepubertal rats) on the ovarian function (and thereby the risk of infertility in case of dysfunction) can be measured by the evaluation of ovarian reserve changes. Ovarian reserve is a commonly used tool to

Abbreviations: AFC, antral follicle count; AMH, anti-Müllerian hormone; DOR, diminished ovarian reserve; FSH, follicle-stimulating hormone; POF, premature ovarian failure; POI, premature ovarian insufficiency.

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https://doi.org/10.1016/j.jpedsurg.2020.05.046

assess ovarian function and it has already been examined in rat models. One of the most common concepts of ovarian reserve views reproductive potential as a function of the number and quality of remaining oocytes. Measures of ovarian reserve have been used to predict diminished ovarian reserve (DOR). Patient with DOR are defined as those more likely to exhibit a poor response to gonadotropin stimulation. These measures are thus surrogates for the clinically important outcomes: pregnancy and live birth. In the case of young patients, ovarian dysfunction has been presented as premature ovarian insufficiency (POI) in most of the studies. Therefore, we have also included the measures of premature ovarian insufficiency in our study. It is important to remember that DOR differs as a clinical diagnosis from POI. DOR is diagnosed by abnormal but not postmenopausal ovarian reserve testing and regular periods. POI (formerly known as POF – premature ovarian failure) is defined as a clinical condition that develops in any adult female at age <40 years and is characterized by the absence of menstrual cycles (amenorrhea) for ≥4 months and two elevated serum follicle-stimulating hormone (FSH) levels in the menopausal range. POI/POF also may result in delayed puberty with FSH levels in the menopausal range. There are various ovarian reserve testing methods but the use of many of them is limited because of lack of validated outcome measures. That is why we concentrated on the recommendations of American Society of Reproductive Medicine as they reviewed most often used ovarian reserve tests and indicated those of the best clinical utility and predictive value. Among many testing options the Society chose anti-Müllerian hormone and antral follicle count as the ones characterized with the best value in evaluating ovarian reserve. In our study, we decided to analyze serum anti-Müllerian hormone (AMH) and follicle-stimulating hormone (FSH) levels (blood tests) and AMH immunostaining with follicle count estimation during histopathological and biochemical examination of the collected tissue [1,7–15].

Based on the former studies, we assumed that ovarian reserve measured by AMH and antral follicle count (AFC) reflects the primordial follicle pool. Nevertheless, some of the recent studies also highlight the possible existence of an ancillary germ cell population coexisting with the primordial follicle pool, the 'reserve' of the reserve. This revolutionary concept is nowadays a topic of intense studies and we should not neglect this aspect when planning a reliable research. In our specific situation of iatrogenic reduction of the primordial follicle pool the possibility to partially replenish the reserve would be of great value. Therefore, we evaluated the whole ovarian morphology by detecting all types of follicles in the collected tissue [16].

1. Material and methods

1.1. General work plan

1.1 Collecting study and control group: female Wistar rats (prepubertal – 26 days of age, 58-100g) - own breeding, two animals in one cage, 12/12 hours light/dark cycle, free access to food and water, no intervention prior to the experiment – Experimental Animal House at Wroclaw Medical University. The groups are presented in Table 1.

a) The study group comprised 76 rats – randomly allocated to one of two groups (two groups of 38 divided into subgroups of 19 – Group B1, B2, BR1, BR2 operated at distinct time).
b) The control group comprised 30 rats also divided into two groups of 15 operated at distinct time (Group C1,C2).

2.1 Surgical procedures:

a) The initial surgery was performed in all rats at the same age. Group B (biopsy) had a unilateral ovarian biopsy performed while group BR (biopsy with resection) had unilateral ovarian biopsy and ovarian resection of the contralateral gonad performed (to imitate the situation of ovarian tumor surgery).
b) The second operation was bilateral ovarian resection in group B in the 8th to 9th week from the initial surgery in half of the group and in the 16th to 17th week in the remaining subjects (group B1 and B2 – assessing correlation between ovarian function restoration and time). In the group BR the second operation was total resection of the remaining ovary also at different times (group BR1 and BR2).
c) The control group had only one operation performed with bilateral ovarian resection in the 8th to 9th week and the 16th to 17th week respectively (group C1 and C2).
d) All surgical procedures were performed through laparotomy. The tissue (only ovarian cortex, not more than half of the ovary) was removed with a scalpel blade no. 11.
e) The second operating procedure was performed on the day of determining the diestrus phase of the estrous cycle according to the cell types observed in the vaginal smear (during seven days between the 8th and 9th week and between the 16th and 17th week from the initial surgery)

3.1 All rats had blood samples collected (lateral saphenous vein puncture) with anti-Müllerian hormone (AMH) and follicle-stimulating hormone(FSH) serum level evaluation at the day of the operation in the study groups and at the beginning of the study and at the day of operation in the control group.

4.1 The collected tissue was examined histologically and biochemically estimating follicle count and AMH immunoexpression.

5.1 All rats from the specific groups were given up for adoption after the completion of the experiments in each group.

1.2. Hormonal profiles

AMH: The assay used in our study (Cusabio, Catalog Number. CSB-E12756h) employs the quantitative sandwich enzyme immunoassay technique.

FSH: The assay used in our study (Cusabio, Catalog Number. CSB-E06869r) employs the competitive enzyme immunoassay technique.

Table 1
Description of the groups.

Group name	C1	C2	B1	B2	BR1	BR2
Number of rats	15	15	19	19	19	19
First intervention	Only blood examination*		Left ovarian biopsy		Left ovarian biopsy and ovarian resection of the contralateral gonad	
Second intervention**	Bilateral ovarian resection		Bilateral ovarian resection		Total resection of the remaining ovary	
Time of the second intervention	8^{th}–9^{th} week	16^{th}–17^{th} week	8^{th}–9^{th} week	16^{th}–17^{th} week	8^{th}–9^{th} week	16^{th}–17^{th} week

* all other groups also had blood examination prior to surgeries
** with blood examination prior to surgery in all groups

1.3. Histopathological examination

Formalin-fixed, paraffin-embedded tissues were cut into 4-μm-thick sections, and five random samples were taken from each ovary to assess follicular activity [8,17].

The samples were stained with hematoxylin–eosin and mounted on Superfrost Plus slides (Menzel Gläser, Braunschweig, Germany). In order to measure the levels of the studied antigen, antibody MIS (Müllerian Inhibiting Substance) (B-11): sc-166752 (Santa Cruz Biotechnology) was applied. The expression of AMH was assessed using the modified semiquantitative immunoreactive score (IRS) scale according to Remmele and Stegner [18,19].

Follicles were counted according to the follicle morphology described as follows: (1) Primordial follicle (2) Primary follicle (3) Preantral follicle (4) Antral follicle. The presence of follicular and stromal degeneration as well as cyst formation was also evaluated. Fig. 1 presents the process of follicle recruitment and selection in human and rat ovaries [20].

1.4. Statistical analysis

Parameters in groups were expressed as median and quartiles or as mean and standard deviation. The statistical significance between independent groups was calculated by one-way analysis of variance (ANOVA), alternatively using the non-parametrical U Mann-Whitney* test, when the variances in groups were not homogeneous (the homogeneity of variance was determined by the Bartelett's test). The statistical significance between dependent groups was calculated by the non-parametrical Wilcoxon# test. The statistical significance between frequencies was calculated by the chi-square test χ^2_{df} with Yates correction with corresponding degree of freedom df (df = (m-1)*(n-1), where m – number of rows, n – number of columns. The relation between two parameters was assessed using correlation analysis and Spearman correlation coefficients were calculated. A p value of less than 0.05 was required to reject the null hypothesis. Software packages: EPIINFO Ver. 7.1.1.14 (02-07-2013). A standardized measure RMSSE (Root Mean Square Standardized Effect) was used to determine the overall effect level in the population.

An additional file shows more information from this section [see Additional file 1].

2. Results

2.1. Hormonal profiles (Table 2, Fig. 2)

When comparing the results between the first and the second blood examination, plasma AMH levels were significantly lower at the second examination in the study group BR2, while they were significantly higher in the study group C2. At the second examination the levels were significantly lower in the group BR1 comparing to C1 and in the group BR2 comparing to B2 and BR1.

When comparing the results between the first and the second blood examination, plasma FSH levels were higher in all groups but the difference was significant in all but one group (BR2). At the second examination the levels were significantly higher in the group B2 comparing to C2 and in the group B2 comparing to BR2.

The level of AMH in the ovarian tissue of the left ovary at the second surgery was significantly higher in the group C2 comparing to C1 ($p = 0.00053$) and B2 ($p = 0.00332$) (Table 2, Fig. 2).

2.2. Ovarian follicular counting (Fig. 3, Table 3)

There were significant differences in the amount of follicles between the groups. Fig. 3 presents a graphical description of the follicle counts of each group. Significant results of a comparison of ovarian follicular counting between the groups are summarized in Table 3.

2.3. Ovarian follicular counting and the hormonal levels (Table 4)

There was a significant positive relation between the amount of the primordial follicles in the right ovary and the plasma AMH level – Spearman correlation $p = 0.00443$ at the second examination. There was also a positive relation between the amount of growing follicles (all together) and the plasma AMH level – Spearman correlation $p = 0.00012$.

There was a significant negative relation between the amount of the atretic follicles in the biopsied ovary and the plasma FSH level - Spearman correlation $p = 0.00442$ at the second examination.

There was a significant positive relation between the amount of the primordial and primary follicles and the level of AMH in the tissue of the left ovary - Spearman correlation $p = 0.0434$ and $p = 0.0126$ respectively.

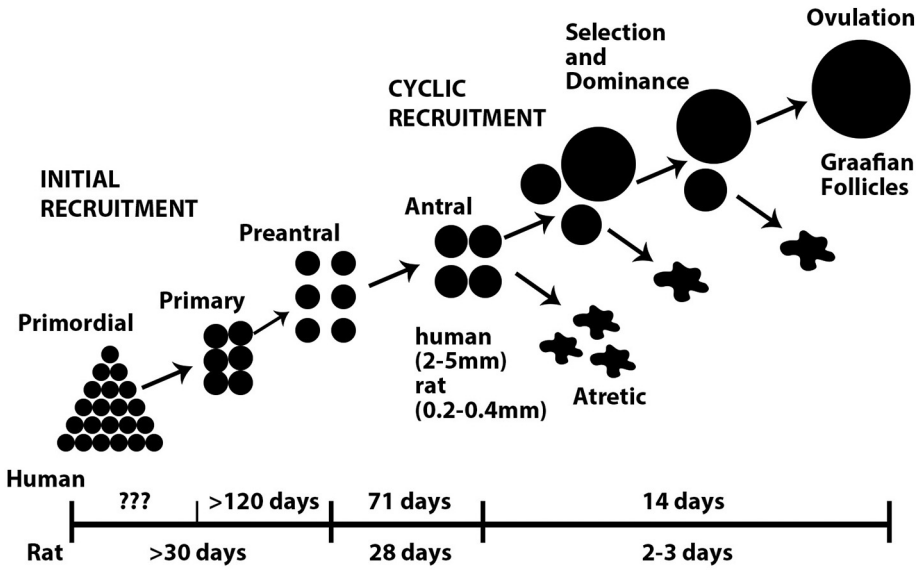

Fig. 1. The process of follicle recruitment and selection in human and rat ovaries. Adapted from reference number [20].

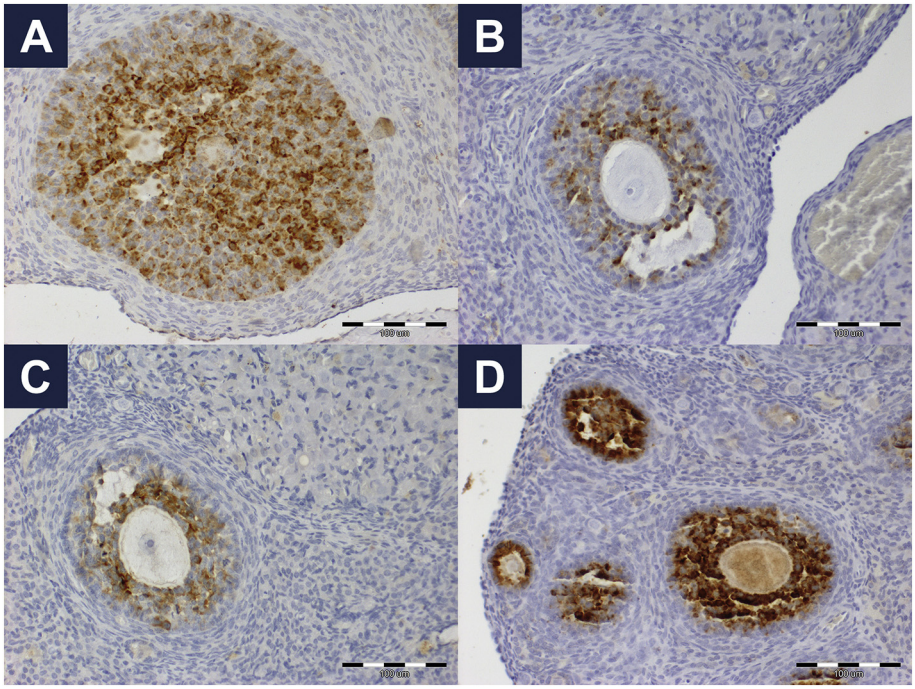

Fig. 2. Cytoplasmic immunoexpression among selected groups. a) Strong cytoplasmic expression of MIS(B-11) in the cells of ovarian follicle in the group C2 (IHC, 200×). b) Moderate cytoplasmic expression of MIS (B-11) in the cells of ovarian follicle in the group C1 (IHC, 200×). c) Moderate cytoplasmic expression of MIS (B-11) in the cells of ovarian follicle in the group B2 (IHC, 200×). d) Strong cytoplasmic expression of MIS(B-11) in the cells of left ovarian follicle (IHC, 200×).

Table 2
Hormonal profiles.

Group number	First examination	Second examination	**p value, Wilcoxon test**
AMH* (median, lower÷upper quartile) (ng/ml)			
- the difference between the examinations (the significant results highlighted in bold)			
C1	11.06 (9.81÷12.16)	12.0 (11.1÷12.8)	0.173
C2	6.85 (4.20÷12.41)	11.4 (9.5÷12.1)	**0.0199**
B1	9.79 (7.10÷12.48)	10.9 (10.0÷11.9)	0.227
B2	12.43 (8.34÷14.37)	11.4 (11.2÷13.0)	0.778
BR1	10.62 (9.16÷12.25)	10.5 (9.7÷11.4)	0.93
BR2	11.22 (10.88÷12.09)	9.64 (8.99÷10.48)	**0.00097**
FSH (median, lower÷upper quartile) (ng/ml)**			
- the difference between the examinations (the significant results highlighted in bold)			
C1	7.83 (7.0÷88.72)	8.59 (8.25÷9.14)	**0.00314**
C2	5.10 (4.70÷7.51)	9.95 (8.75÷10.58)	**0.00066**
B1	7.34 (6.65÷8.52)	9.02 (8.33÷9.79)	**0.00170**
B2	8.30 (5.92÷9.40)	10.87 (10.09÷11.95)	**0.00021**
BR1	8.00 (7.11÷9.22)	8.89 (8.33÷10.20)	**0.0401**
BR2	8.25 (7.90÷8.97)	9.61 (7.62÷10.22)	0.198
First examination	**p Value, Mann-Whitney *U* test**	Second examination	**p Value, Mann-Whitney *U* test**
AMH*			
- the significant difference between the groups			
C2 vs B2	0.0153	C1 vs BR1	0.0153
C2 vs BR2	0.025	B2 vs BR2	0.00003
		BR1 vs BR2	0.0462
FSH**			
- the significant difference between the groups			
C1 vs C2	0.00494	C1 vs C2	0.0329
C2 vs B2	0.0138	B1 vs B2	0.00004
C2 vs BR2	0.00022	C2 vs B2	0.0138
		B2 vs BR2	0.00054
AMH* tissue			**p Value, Mann-Whitney *U* test**
- the significant difference between the groups			
		C2 vs B2	0.00332
		C1 vs C2	0.00053

* AMH: anti-Müllerian hormone; **FSH: Follicle-stimulating hormone.

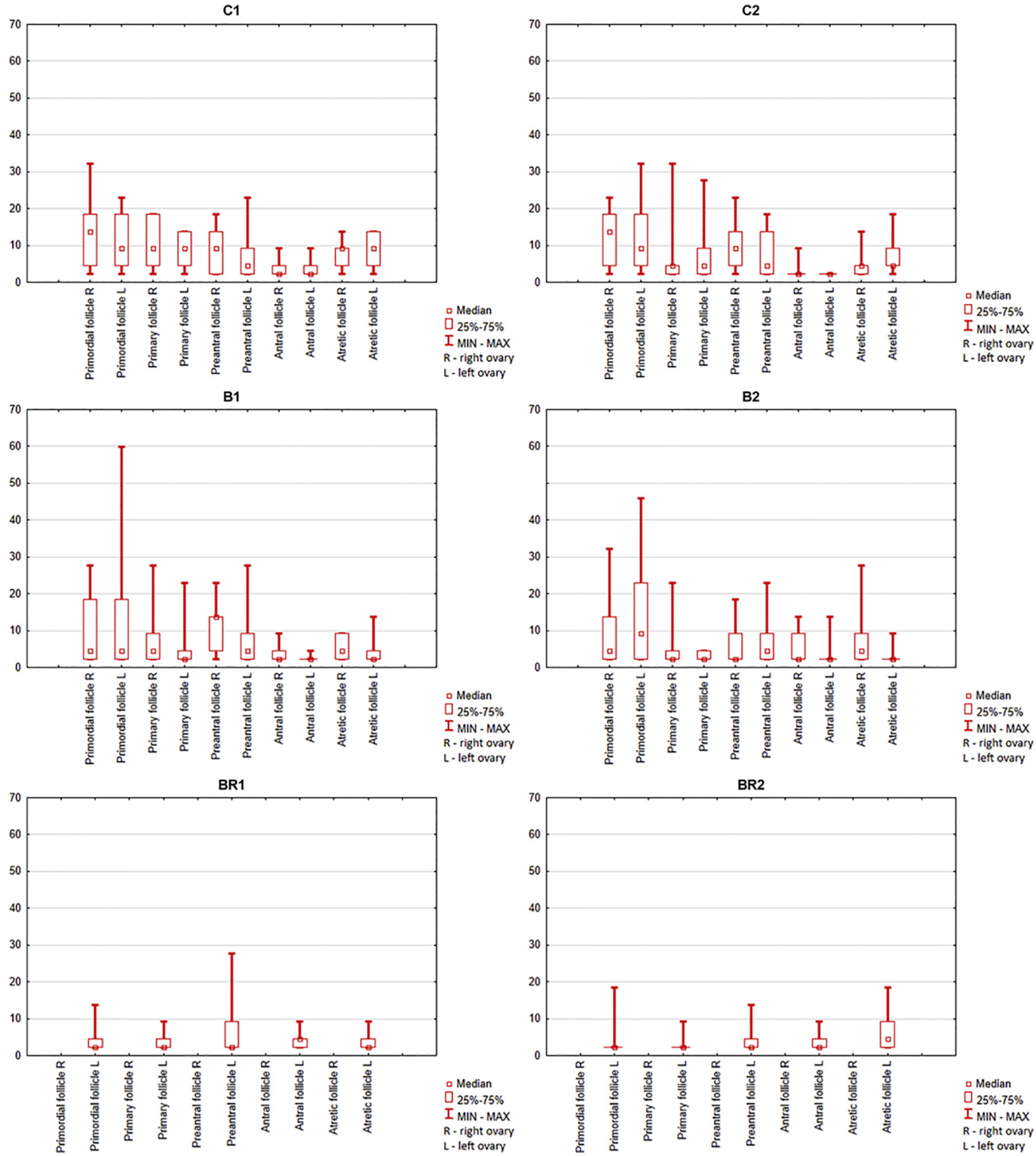

Fig. 3. Graphical description of the follicle counts among groups.

2.4. Irregular estrous cycle, cyst formation or follicular and stromal degeneration (Table 5)

There were some differences between the animals which had irregular cycles or whose ovaries presented one of the above mentioned features and the other rats. The amount of primordial, primary and atretic follicles in the biopsied ovary at the second examination was less numerous in these rats. The described events happened more often in the group BR1 comparing to the other groups and less common in the group C1 ($\chi^2_5 = 25.7$, $p = 0.00010$). Irregular cycles analyzed alone were more frequent in the group B1 comparing to the other groups and less common in the group C1 and B2 ($\chi^2_5 = 11.3$, $p = 0.045$).

Table 3
Significant differences in the amount of follicles between the groups at the second examination.

Compared groups and values (median; lower ÷ upper quartile)		p Value, Mann-Whitney *U* test
Primordial follicles (median; lower ÷ upper quartile)		
C1 (9.2; 4.60 ÷ 18.40)	BR1 (2.3; 2.30 ÷ 4.60)	0.00019
C2 (9.2; 4.60 ÷ 18.40)	BR2 (2.3; 2.30 ÷ 2.30)	0.00050
B2 (9.2; 2.30 ÷ 2.30)	BR2 (2.3; 2.30 ÷ 2.30)	0.0119
Primary follicles (median; lower ÷ upper quartile)		
C1 (9.2; 4.60 ÷ 13.80)	B1 (2.3; 2.30 ÷ 4.60)	0.0138
C2 (4.6; 2.30 ÷ 9.20)	B2 (2.3; 2.30 ÷ 4.60)	0.0111
C1 (9.2; 4.60 ÷ 13.80)	BR1 (2.3; 2.30 ÷ 4.60)	0.000271
C2 (4.6; 2.30 ÷ 9.20)	BR2 (2.3; 2.30 ÷ 2.30)	0.00895
Preantral follicles (median; lower ÷ upper quartile)		
B1 (4.6; 2.30 ÷ 9.20)*	B2 (2.3; 2.30 ÷ 4.60)*	0.0462
Antral follicles (median; lower ÷ upper quartile)		
BR1 (4.6; 2.30 ÷ 4.60)	B1 (2.3; 2.30 ÷ 2.30)	0.00905
Atretic follicles (median; lower ÷ upper quartile)		
C1 (9.2; 4.60 ÷ 13.80)	B1 (2.3; 2.30 ÷ 4.60)	0.00120
C1 (9.2; 4.60 ÷ 13.80)	BR1 (2.3; 2.30 ÷ 4.60)	0.00182
C2 (4.6; 4.60 ÷ 9.20)	B2 (2.3; 2.30 ÷ 2.30)	0.00067
BR2 (4.6; 2.30 ÷ 9.20)	B2 (2.3; 2.30 ÷ 2.30)	0.0168

* Right ovary.

3. Discussion

Numerous studies focus on the negative influence of ovarian surgery on the ovarian function. Some surgical interventions like ovarian wedge resection, laparoscopic ovarian drilling, resection of ovarian endometrioma or another benign cyst caused a decrease of ovarian reserve measures or earlier menopause [15,21–23]. However, the studies concerned previously affected gonads and reproductive age population. Hence our study is unique as, to our knowledge, there is only one research study concerning a similar clinical situation in healthy ovaries and a prepubertal patients group. Nevertheless, in the study by Abrir et al. only anti-Müllerian hormone levels were measured in serum before and after partial oophorectomy for cryopreservation and the blood samples were collected 24 h after the procedure [23].

Recent studies suggest extra caution when interpreting AMH values before the age of 25. AMH may present significant fluctuations throughout the menstrual cycle, especially in young women. Nevertheless, its many advantages make the evaluation of this marker very promising and it is widely adopted in many studies concerning children. AMH compared to FSH may be an earlier marker of reduced ovarian reserve. It is also possible that pretreatment (pre-surgery) AMH has prognostic significance. Wong and Anderson indicated that AMH pre- and post-surgery is useful in assessing the degree of damage to the ovary (described as DOR). Still, the implications of low AMH on natural fertility and reproductive lifespan are not clear. However, there is a general agreement about lower pregnancy rates of women with very low serum AMH in the case of assisted reproduction. Age-independent standardization of AMH values may help compare ovarian reserves among women at different ages and a nomogram integrating serum AMH as a stimulation protocol is useful for avoiding poor and/or hyper-responses [14,24–29].

Recent studies suggest that primordial follicle activation involves a balance between activation-promoting and activation-inhibiting factors. It is also possible that AMH alters the relative rate of primordial follicle activation in a context-dependent manner to maximize lifetime reproductive opportunity. Furthermore, the control of follicle activation may be complex and AMH seems to play a putative function in this process [24,30].

In our study, AMH plasma levels were significantly lower at the second examination in the rats which had unilateral ovarian biopsy and ovarian resection of the contralateral gonad performed at the first surgery and longer follow-up (group BR2). It confirms the observation of other authors reporting AMH level decrease after ovarian surgery and the concept that it reflects the size of primordial follicle pool. However, our results showed a significant decrease only in the group with longer follow up. In the group with shorter time of observation and in rats with a small amount of removed tissue (the subgroups with only biopsy performed) the levels were higher comparing to the first examination but the increase was not statistically significant [15,24,31].

The FSH plasma levels were higher in all groups but the difference was significant in all but one group which was again the group BR2. According to many studies FSH levels are increased in case of diminished ovarian reserve. In this respect we should expect to obtain higher levels in the group with more aggressive surgery performed. However, the literature reviewed also revealed studies where the FSH decreased in the injured ovaries. As indicated by the authors, it can have many causes including a pathological central stimulation due to irregular cycles, alteration in normal functions of the hypothalamic-pituitary axis, testing hormonal levels irrespective of estrous cycle or suppression by the unexpected rise of estradiol level. In our material, the level of FSH was not significantly different in the rats with irregular cycle, cyst formation or ovarian degeneration ($p = 0.657$). It is worth mentioning that many studies test only post-treatment hormonal levels, which makes the comparison of the data challenging [14,25,32–35].

Regarding the relation between the follicular count and hormonal levels, there was a significant relation between the amount of the primordial follicles and the level of AMH in the ovarian tissue as well as plasma AMH. This corroborates with other authors' results [15,24,31].

As for follicular count, it is not surprising that the number of primordial follicles was smaller in the rats which had more extensive surgery. However, in our study the difference was noted only in the groups with the removal of the contralateral ovary in addition to biopsy. It is worth mentioning that in contrast with some other studies, atretic follicles were not increased in these animals comparing to the control group. This may indirectly indicate some mechanism of ovarian tissue protection in the case of severe injury [36].

Our study poses limitations. Since the rats in our study were in a similar habitat and nutritional status, we assumed that they should have similar ovarian reserve. Still, there were some differences in hormonal levels at the initial examination between the groups. Some of the rats had irregular cycles. This group was not large enough to test the possibility of differences in the results in this respect only. During the biopsy procedure we were removing similar amount of ovarian tissue, nevertheless, it was not exactly the same as we did not measure its weight. We are not able to test estradiol levels either, although it is indicated as a measure of premature ovarian insufficiency in prepubertal patients. The amount of blood we could extract from each animal was not sufficient for testing three hormones. Basal estradiol alone should not be used to screen for DOR. The test has value probably only as an aid to

Table 4
Significant relations between ovarian follicular counting and the hormonal levels at the second examination.

Hormone tested	Follicle type	Ovary	p Value	R (Spearman coefficient)
AMH*	primordial follicle	right	0.00443	0.34
AMH	growing follicles	left and right	0.00012	0.37
AMH tissue	primordial follicle	left	0.0434	0.20
AMH tissue	primary follicle	left	0.0126	0.25
FSH**	atretic follicle	left	0.00442	-0.27

* AMH: anti-Müllerian hormone; **FSH: Follicle-stimulating hormone.

Table 5
Significant differences between rats with or without special features.*

Relation tested	Rats with special features (median, lower÷upper quartile or mean±S.D.)	Rats without special features (median, lower÷upper quartile or mean±S.D.)	p Value
primordial follicles in the left ovary	2.3 (2.30÷4.60)	9.2 (2.30÷18.40)	0.0130[1]
primary follicles in the left ovary	2.3 (2.30÷4.60)	2.3 (2.30÷9.20)	0.0491[2]
atretic follicles in the left ovary	2.3 (2.30÷4.60)	4.6 (2.30÷9.20)	0.00094[2]
AMH** in the tissue of the left ovary	2.59±2.93	3.79±3.55	0.0747[1]

* Irregular estrous cycle, cyst formation or follicular and stromal degeneration.
** AMH, anti-Müllerian hormone.
[1] ANOVA.
[2] Mann-Whitney *U* test.

correct interpretation of a "normal" basal serum FSH value. Nevertheless, to standardize our study group we decided to perform blood tests and operating procedures simultaneously during the diestrus phase of the rat estrous cycle. However, we were not able to detect unexpected rise of estradiol level [1,24].

It is undeniable that overall reproductive health is an important measure of the general health and social well-being. Cryopreservation and further autotransplantation of ovarian tissue already proved to restore fertility in a pediatric population. However, in a study by Sullivan-Pyke et al. the parents who declined ovarian tissue cryopreservation regarded it as a good idea but the risks concerning biopsy caused their refusal [37,38]. Evaluating the risks carried by ovarian biopsy and ovarian cryopreservation procedure will provide medical practitioners with knowledge facilitating treatment decisions and follow-up of patients. It was both a cost and time-sparing opportunity to obtain highly valuable data that would not be possible with human-based research. The research based on measuring the fecundability by pregnancy as a final outcome also requires long-term follow-up. A goal difficult to achieve especially in a pediatric population [15,24,30,31]. The study provided data that are scientifically accepted surrogates for pregnancy outcome. Nevertheless, we are planning our next study in a similar rat model to measure the ovarian function by means of pregnancy rate. The proposed scientific methods may be a useful tool in the evaluation of ovarian fertility potential in children and form an objective base for a future prospective long-term study in humans.

4. Conclusions

The results we obtained indicate that ovarian reserve seemed to be unaffected in the case of biopsy alone. A significant decrease in the number of primordial and primary follicles as well as AMH levels noted only in the case of biopsy combined with contralateral oophorectomy indicate complex regulation of ovarian reserve, as proposed in the previously mentioned studies. Biopsy itself might not be harmful if done properly but we should know its possible negative effects in the case of a single remaining ovary. Until there is more reliable data on AMH functions, we should consider it an important measure and include its evaluation in children before and after treatments potentially affecting ovaries.

Supplementary data to this article (Additional file 1 and ARRIVE Guidelines Checklist) can be found online at https://doi.org/10.1016/j.jpedsurg.2020.05.046.

Declarations

Ethical approval

The study was approved by the Local Institutional Animal Care and Use Committee, permission no. 99/2017- available on request.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Grant for Young Investigators – Wroclaw Medical University, grant no. STM.C030.17.057 – financial support for the conduct of the research. The funding body had no involvement in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Authors' contributions

JŁ, MB and RC – substantial contributions to the conception and design of the work, the acquisition, analysis and interpretation of data for the work; and drafting the work as well as revising it critically for important intellectual content; and final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

AS – substantial contributions to the acquisition, analysis and interpretation of data for the work; and drafting the work as well as revising it critically for important intellectual content; and final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

MN – substantial contributions to the conception and design of the work, analysis and interpretation of data for the work; and drafting the work as well as revising it critically for important intellectual content; and final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Acknowledgements

We thank the team of Experimental Animal House at Wroclaw Medical University and Aleksandra Mądry for invaluable help, great professionalism and support.

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