Published in final edited form as:

J Pediatr Surg. 2012 June ; 47(6): 1272–1279. doi:10.1016/j.jpedsurg.2012.03.038.

Assessing gonadal function after childhood ovarian surgery

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Abstract

Purpose—We aimed to assess the late effects of ovarian salvage or oophorectomy on gonadal function and fertility as measured by menstrual regularity.

Methods—We performed a 10-year retrospective review of females aged 20 years or younger who required surgery to treat an ovarian disorder. A mail survey was distributed to these patients to evaluate the effects of ovarian surgery on menarche, menstrual regularity, and pregnancy.

Results—A total of 180 females had surgery to treat an ovarian disorder. Eighty-six of these underwent unilateral oophorectomy (48%), whereas 94 (52%) had an ovary sparing procedure. Eighty-one patients (45%) returned completed surveys. Of the respondents, 44 had oophorectomy, and 37 had ovarian salvage. Ages of menarche were similar between surgical groups. Symptoms of menstrual irregularity differed most significantly according to painful menses (oophorectomy, 27.3%; salvage, 59.5%; $P < .04$). Interestingly, continuation of regular menses after surgery was higher in the oophorectomy group (oophorectomy, 70%; salvage, 15%; $P = .013$).

Conclusions—Unilateral oophorectomy does not appear to impair late gonadal function when compared with ovarian salvage. Surprisingly, oophorectomy appears to maintain more normal ovarian activity as estimated by menstrual regularity. Oophorectomy may be performed without apparent adverse effect on gonadal activity.

Keywords

Oophorectomy; Ovarian salvage; Fertility; Ovarian function; Menstrual irregularity; Children

Surgical intervention is often required for a wide variety of childhood ovarian and adnexal pathologies. Although relatively rare, adnexal torsion and ovarian masses are important disorders of the reproductive tract and comprise the majority of ovarian and fallopian tube disturbances treated by pediatric surgeons. Ovarian torsion accounts for up to 2.7% of all cases of acute abdominal pain in children [1]. Ovarian masses occur with an incidence of 2.6 per 10,000 cases per year in children [2] and may be nonneoplastic, including simple and complex cysts, or neoplastic. Neoplastic processes include both benign tumors, such as mature cystic teratomas as well as highly malignant tumors, which constitute approximately 1% of all childhood malignancies [3].

Although operative intervention continues to be the mainstay of therapy for ovarian pathologies, uncertainty remains regarding the late effects of different surgical options, such as unilateral salpingo-oophorectomy or ovarian salvage, on subsequent gonadal function. Current literature recommends conservative treatment and preservation of ovarian tissue whenever possible in young girls to ensure normal development of female characteristics and future fertility [4]. Detorsion of ischemic adnexa without resection (ie, ovarian salvage) is now considered safe and effective for most idiopathic ovarian torsion cases [1,5-8], and observation of small, asymptomatic simple ovarian cysts is recommended as initial management [9]. Furthermore, ovary-sparing operations have been advocated for all patients having clinically benign-appearing masses and normal tumor markers [10-13]. In contrast, for malignant or premalignant conditions, the primary goal of surgery is to eradicate the cancer and to reduce its risk of recurrence while balancing deleterious effects on residual ovarian function. Resection of an ischemic-appearing ovary may be necessary if concern exists regarding the possibility of an associated malignancy or other indeterminate mass; the theoretical risk of thromboembolism after detorsion; and the unknown sequelae, such as infertility or ectopic pregnancy, of leaving frankly necrotic ovarian tissue or fallopian tube in situ [1,6]. Although current treatment algorithms attempt to balance future ovarian function with an increase in the certainty of a cure, evidence now suggests that women having a single ovary are still able to maintain relatively normal fertility, even after having been treated for an ovarian malignancy [14]. The purpose of this study was to compare the late effects of ovarian salvage or unilateral oophorectomy on menstrual regularity as a measure of gonadal function and ultimate fertility.

1. Methods

1.1. Cohort

International Statistical Classification of Diseases and Related Health Problems, Ninth Revision, codes were used to identify patients from birth to 20 years of age who were surgically managed for an ovarian condition at an academic health care facility consisting of an adult hospital and a freestanding children's hospital between August 2000 and August 2010. One hundred eighty patients were included in the analysis. A single researcher (AZ) abstracted qualifying records for demographics, age at diagnosis, surgical procedure(s) performed, and pathology.

1.2. Survey

To evaluate the late effects of either unilateral oophorectomy or ovarian salvage in childhood on subsequent gonadal function, we conducted a mail survey of all 180 study patients. A panel of researchers developed an 11-item survey to capture effects on the onset of menarche and puberty, history and symptoms of menstrual irregularity, age at start of menstrual irregularity, need for hormone regulation, and ability to achieve pregnancy (Fig. 1). The revised survey was then distributed to the entire study cohort along with a letter of intent and appropriate assent and consent forms. In cases where patients were younger than 18 years at the time of follow-up, parents or legal guardians were provided the option of completing the survey. The institutional review board approved all aspects of the study (no. 091112).

1.3. Data management

Data from the chart review and survey responses were compiled into a computer database, and descriptive univariate analyses were performed. Continuous data were compared using the nonparametric Wilcoxon rank sum test. Proportions were compared using the χ^2 test or Fisher exact test in instances where cell values were less than 5. Significance was set to $P < .05$. All statistical tests were performed using STATA 11.2 (2009 StataCorp LP, College Station, TX).

2. Results

Within our academic center, a total of 180 patients (mean age, 11.5 years; range, 4 days to 20 years) were identified as having had an operation to treat an ovarian disorder during the study period. Eighty-six females had unilateral oophorectomy with or without salpingectomy, and 94 had ovary-sparing procedures, including partial oophorectomy, cystectomy, salpingectomy only, tumor enucleation, detorsion, oophoropexy, or drainage. Median follow-up time was 40.5 months for the oophorectomy group and 29 months for the ovarian salvage group.

The survey was mailed to all 180 patients, and 81 completed forms were returned (45% response rate). Characteristics of respondents and nonrespondents only differed by race; those responding were predominantly white ($n = 67$, 81.5%) (Table 1). All other characteristics were similar between response groups. Mean age of respondents was 138 months (interquartile range, 114-173 months) at diagnosis and 178 months (interquartile range, 147-219 months) at survey. Mean age of nonrespondents was 137 months (interquartile range, 98-191 months) at diagnosis and 181 months (interquartile range, 128-232 months) at survey. Simple cyst, benign tumor, and torsion owing to mass were the most common diagnoses in both groups. Unilateral salpingo-oophorectomy, oophorectomy, and cystectomy were the most common procedures.

Of the respondents, 44 had unilateral oophorectomy, and 37 had an ovary-sparing procedure. Tables 2 and 3 compare the 2 groups. As expected, a diagnosis of complex or simple cyst (32.4% and 48.7%, respectively) was notably higher in the group with ovary-sparing procedures ($P = .02$), whereas a diagnosis of benign or malignant tumor (54.6% and 9.1%, respectively) was more common in the group having an oophorectomy ($P < .0001$). The median age at onset of menarche (12 years) was similar between the 2 groups. No difference in achievement of normal menarche, defined as menarche occurring between ages 10 and 15 years, was observed across surgical groups. Painful menses were significantly more common in females having an ovary-sparing procedure (59.5%) when compared with females having a unilateral oophorectomy (27.3%; $P = .003$). A history of menstrual irregularity, defined as cycles longer or shorter than 28 to 30 days, abnormally heavy or light bleeding, and/or severe pain with menses, was reported in 70% of the patients having ovary-sparing procedures relative to 50% of the patients having oophorectomies who were postmenarche at time of survey ($P = .181$). Of 4 women who had a unilateral oophorectomy in childhood and attempted to become pregnant, 3 reported successful conception and delivery, whereas none in the salvage group had attempted to become pregnant.

Twenty-three of the survey respondents who underwent ovary-sparing procedures and 19 of the respondents who underwent oophorectomies were postmenarche at the time of surgery. Fig. 2 compares the menstrual cycles of these 2 surgical groups before and after surgery. The number of patients having regular menstrual cycles before surgery and who continued to be regular after surgery was significantly higher in the oophorectomy group with 7 (70%) of 10 patients noting continued menstrual regularity, whereas only 2 (15%) of 13 patients reported regular cycles in the ovarian salvage group ($P = .013$). All patients who reported irregular cycles before surgery remained irregular after surgery.

3. Discussion

Although current studies recommend conservative treatment of pediatric ovarian disorders to ensure future fertility, a review of the literature yielded little information directly comparing the late effects of ovary salvage vs oophorectomy on subsequent fertility. Studies in adults suggest an association between type of ovarian surgery and subsequent fertility as measured by achievement of pregnancy. In a series reported by Yinon et al [15] comparing the results of cystectomy vs oophorectomy for borderline epithelial tumors, the fertility rate was lower in patients treated with the gonadal sparing cystectomy. Perhaps leaving an injured ovary in some way hinders fecundity. On the other hand, in a separate study of borderline tumors comparing unilateral salpingo-oophorectomy and contralateral cystectomy or bilateral cystectomies in patients treated for bilateral tumors, the authors showed a higher cumulative pregnancy rate in patients having undergone bilateral cystectomies compared with unilateral oophorectomy and contralateral cystectomy [16]. These results suggest that cystectomy should be preferred, if technically feasible. However, this latter trial was limited by a small number of patients (15 in the cystectomy group and 17 in the cystectomy with oophorectomy group).

Because achievement of pregnancy is an unrealistic outcome variable in the pediatric population, we felt that an examination of menstrual events would be an appropriate surrogate assessment of gonadal function in females who required ovarian surgery. Although menstrual function may be perceived as an inaccurate indicator of future fertility, early pioneering studies on endometrial dating by Rock and Bartlett [17] revealed a significant correlation between ovulatory function and regularity of the menstrual interval and flow pattern. A more recent article documents a relationship between 30- to 31-day menstrual cycles and 5-to 7-day bleeding periods and fecundity [18]. In addition, Gardner [19] found that women experiencing dysmenorrhea and cycle irregularity in the first few years postmenarche were at risk for having poor gynecological health later in life. Although painful menses with no pelvic pathology and normal ovulatory cycles (primary dysmenorrhea) is not linked to infertility [20], dysmenorrhea in the context of established ovarian pathology and irregular cycles as well as previous surgery is important to consider when discussing gonadal function and fecundity. Secondary dysmenorrhea is highly correlated with gynecological pathologies that have well-documented effects on fertility [21]. Disease processes such as endometriosis, ovarian/pelvic adhesions, and large hormone-secreting cysts have been shown to disrupt folliculogenesis, reduce fertilization potential of oocytes as well as inhibit oocyte release and transport [22-24]. In addition, the likelihood of developing these disorders appears to increase with pelvic surgery [21,25], and recurrence

rates can be high even after surgical intervention [9,26,27]. Menstrual cycle irregularity and secondary dysmenorrhea are likely associated with reduced fertility and impaired gonadal function and are certainly associated with patient discomfort and anxiety.

When we compared females with ovary-sparing procedures to females with unilateral oophorectomies, there was a notable increase in menstrual irregularity and painful menses in the ovarian salvage group. No difference between these groups was observed in achievement of normal menarche or age at onset of menarche. In our cohort, oophorectomy did not appear to delay or impair long-term hormonal function when compared with ovarian salvage. Lass [14], who performed a comprehensive literature review of reproductive performance in adult women with a single ovary, also concluded that absence of an ovary does not reduce fertility potential. However, women have no compensatory mechanism for the loss of 1 ovary, and because the number of primordial follicles in the ovary is finite, these women may have a shorter reproductive lifespan [14].

An alternative explanation for the increase in menstrual irregularity observed in the ovarian salvage group may be differences in initial underlying disease etiology and pathology between the 2 surgical groups. The salvage group had more cystic disease, which is hormonally driven and, hence, presumably more likely to affect menses, as opposed to the oophorectomy group, which predominantly had tumors. However, when we followed the menstrual cycles of females who were postmenarche at the time of surgery from preoperative to postoperative, our data showed similar percentages of irregularity in both groups before surgery and a marked increase in irregularity in the salvage group after surgery. Interestingly, continuation of menstrual regularity from preoperative to postoperative was significantly higher in the oophorectomy group vs the ovary salvage group. If initial underlying pathology were solely responsible for the differences in menstruation seen in the 2 surgical groups, we would predict a higher percentage of irregularity in the salvage group before surgery and similar menstruation patterns across groups once the offending disease had been eradicated. Instead, the opposite trend was observed. Perhaps the presence of an altered ovary somehow disorders menses in perimenarchal women. Although the effects of pathology should not be discounted, oophorectomy may surprisingly increase the chances of preserving normal ovarian activity.

Interpretation of our study results is limited by the retrospective study design coupled to a survey. Retrospectively obtained data are limited to information already recorded within the record for purposes of clinical care. However, we attained a respectable 45% response rate to the survey. Notably, those that responded were primarily of white race. Respondents also had oophorectomies in greater proportion. These observations may indicate differential response rates within subsets and could bias our findings. Symptomatic patients may have responded in greater proportion too. These factors may have influenced the differential response rates observed yet, if true, should have increased the amount of respondents reporting painful menses in those who had oophorectomies. Differential response rates did not otherwise impact the survey in substantive ways. Nevertheless, we did not find any significant differences between respondents and nonrespondents and so conclude that this subset is representative of the entire cohort. In addition, as with all surveys, it is possible that

the language of each question biased a patient's or guardian's response in a manner that could have altered the study findings.

3.1. Conclusions

This study should be reassuring to patients, guardians, and care providers, having or treating an ovarian condition, which requires unilateral oophorectomy, in that loss of a single ovary does not appear to adversely affect gonadal function, as estimated by effects on menstrual regularity, nor does it appear to compromise future fertility. However, we continue to advocate the conservative approach to spare ovaries whenever possible and feasible. Notably, the sparing of ovaries in this population of young females may result in painful and/or irregular menses, which will be beneficial knowledge for patients, guardians, and care providers. To the best of our knowledge, this study is the first to attempt to address the controversy of late effects of childhood ovarian surgery on subsequent ovarian function. Larger prospective, long-term follow-up studies are needed to shed more light on this important topic.

Acknowledgments

The authors would like to recognize the generous support of the NIH Clinical and Translational Science Awards CTSA program (VICTR#1142).

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This survey will take approximately 10 minutes. You will be asked several questions about fertility that are personal in nature, such as menstrual function. Because of the range of ages of participants, some questions may not seem appropriate for you. You do not have to answer every question.

Questions:

1. Have you ever had a period? ____ No ____ Yes
2. If yes, at what age did you have your first period? _____
3. Have you ever experienced irregular menses (periods)? ____ No ____ Yes
If yes, have you ever had
 - a. menses cycles longer than 28-30 days ____ No ____ Yes
 - b. menses cycles shorter than 28-30 days ____ No ____ Yes
 - c. abnormally heavy bleeding ____ No ____ Yes
 - d. abnormally light bleeding ____ No ____ Yes
 - e. severe pain with menses ____ No ____ Yes
 - f. other (please explain) _____
 - g. At what age did you start having irregular menses? _____
 - h. Do you have irregular menses now? ____ No ____ Yes
4. Did you ever experience any early puberty? Early puberty includes any puberty changes before the age of 9. Some examples of puberty changes are breast development, pubic hair, or menses. ____ No ____ Yes
If yes, how old were you when you first developed puberty changes? _____
If yes, what changes of puberty did you experience? _____
5. Did you ever experience any delayed puberty? Delayed puberty includes no breast development by 13 years of age, or no menses by 3 years after breast development, or no menses by age 16. ____ No ____ Yes
If yes, what changes did you not experience? _____
6. At any point in time did your menses stop for > 6 months? ____ No ____ Yes
If yes, at what age did your menses stop? _____
If yes, for how long did your menses stop? _____
If yes, did your menses ever return to regular 28-30 day cycles? _____
7. Do you take any hormone medicines (for example, birth control pills)? _____
If yes, do you take hormone medicines
 - a. to help regulate menses? ____ No ____ Yes
 - b. for hormone replacement therapy? ____ No ____ Yes
 - c. to help become pregnant? ____ No ____ Yes
 - d. other (please explain) _____

The following questions pertain to pregnancy:

8. Have you ever been pregnant? ____ No ____ Yes
If yes, how many times have you been pregnant? _____
How many live births have you had? _____
At what age(s) were you pregnant? _____
9. Have you ever tried to become pregnant? ____ No ____ Yes
10. Have you ever tried to become pregnant and could not? ____ No ____ Yes
If yes, at what age did you try to become pregnant? _____
11. Have you used fertility treatments? ____ No ____ Yes
If yes, have you ever used
 - a. fertility drugs (such as clomiphene or gonadotropins) ____ No ____ Yes
 - b. artificial insemination ____ No ____ Yes
 - c. in vitro fertilization ____ No ____ Yes
 - d. donor eggs and embryos ____ No ____ Yes

Fig. 1.
Survey characterizing the effects of ovarian surgery on menstruation and pregnancy.

Fig. 2.

Effects of ovarian salvage vs oophorectomy on the menstrual cycles of patients who were postmenarche at the time of surgery.

Table 1

Comparison of characteristics of respondents vs nonrespondents

	<u>Nonrespondents</u>	<u>Respondents</u>	<u>P</u>
	<u>n = 99</u>	<u>n = 81</u>	
Ethnicity			.005
Unspecified	0% (0)	7.4% (6)	
White	72.7% (71)	81.5% (67)	
Black	15.2% (15)	6.2% (5)	
Hispanic	9.1% (9)	4.9% (4)	
Asian	1% (1)	0% (0)	
Middle Eastern	2% (2)	0% (0)	
Age at diagnosis (IQR)	137.3 (96-191)	138 (114-173)	.937
Age at survey (IQR)	181.4 (128-232)	178.1 (147-219)	.5038
Ovary sparing			.112
Yes	57.6% (57)	45.7% (37)	
No	42.4% (42)	54.3% (44)	
Diagnosis ^a			
Normal ovaries	3% (3)	1.2% (1)	.628
Simple cyst	49.5% (49)	35.8% (29)	.065
Complex cyst	14.1% (14)	21% (17)	.226
Benign tumor	20.2% (20)	32.1% (26)	.069
Malignant tumor	2% (2)	4.9% (4)	.411
Torsion owing to mass	20.2% (20)	25.9% (21)	.362
Idiopathic torsion	14.1% (14)	17.3% (14)	.563
Incarcerated ovary owing to hernia	2% (2)	0% (0)	.502
Procedures ^a			
Exploratory	4% (4)	2.5% (2)	.692
Salpingo-oophorectomy	21.2% (21)	34.6% (28)	.045
Oophorectomy	21.2% (21)	19.8% (16)	.81
Partial oophorectomy	3% (3)	4.9% (4)	.702
Cystectomy	28.3% (28)	27.2% (22)	.867
Salpingectomy	3% (3)	9.9% (8)	.067
Mass resection	2% (2)	3.7% (3)	.659
Detorsion	11.1% (11)	14.8% (12)	.459
Oophoropexy	10.1% (10)	7.4% (6)	.528
Drainage	17.2% (17)	16.1% (13)	.841
Hernia Repair	2% (2)	0% (0)	.502

IQR indicates interquartile range.

^a A person may have more than 1 diagnosis and more than 1 procedure. χ^2 Was used when all expected cell counts are more than 5. Fisher's Exact test was used when expected cell counts are less than < 5. Wilcoxon rank sum test was used to compare continuous variables.

Table 2

Comparison of characteristics of respondents with ovary-sparing procedures vs respondents with oophorectomies

	<u>Ovary sparing</u>	<u>Oophorectomy</u>	<u>P</u>
	<u>n = 37</u>	<u>n = 44</u>	
Ethnicity			.744
Unspecified	8.1% (3)	6.8% (3)	
White	83.8% (31)	79.6% (35)	
Black	2.7% (1)	9% (4)	
Hispanic	5.4% (2)	4.6% (2)	
Age at diagnosis (IQR)	140 (121-179)	136.4 (102-173)	.778
Age at survey (IQR)	173.5 (163-219)	182 (141-223)	.754
Diagnosis ^a			
Normal ovaries	2.7% (1)	0% (0)	.457
Simple cyst	48.7% (18)	25% (11)	.027
Complex cyst	32.4% (12)	11.4% (5)	.02
Benign tumor	5.4% (2)	54.6% (24)	.000
Malignant tumor	0% (0)	9.1% (4)	.121
Torsion owing to mass	21.6% (8)	29.6% (13)	.418
Idiopathic torsion	13.5% (5)	20.5% (9)	.411
Procedure ^a			
Exploratory	5.4% (2)	0% (0)	.206
Salpingo-oophorectomy	0% (0)	63.6% (28)	.000
Oophorectomy	0% (0)	36.4% (16)	.000
Partial oophorectomy	10.8% (4)	0% (0)	.025
Cystectomy	46% (17)	11.4% (5)	.000
Salpingectomy	21.6% (8)	0% (0)	.001
Mass resection	5.4% (2)	2.3% (1)	.59
Detorsion	27% (10)	4.6% (2)	.005
Oophoropexy	16.2% (6)	0% (0)	.007
Drainage	24.3% (9)	9.1% (4)	.063

^a A person may have more than 1 diagnosis and more than 1 procedure. χ^2 Was used when all expected cell counts are more than 5. Fisher's Exact test was used when expected cell counts are less than 5. Wilcoxon rank sum test was used to compare continuous variables.

Table 3

Comparison of menstrual regularity and pregnancy in respondents with ovary-sparing procedures vs respondents with oophorectomies

	<u>Ovary sparing</u> n = 37	<u>Oophorectomy</u> n = 44	<i>P</i>
Age at menarche (among those postmenarche)	12 (11-13)	12.2 (12-13)	.256
Menarche at time of surgery			.089
Premenarche	37.8% (14)	56.8% (25)	
Postmenarche	62.2% (23)	43.2% (19)	
Menarche at time of survey			.187
Premenarche	18.9% (7)	31.8% (14)	
Postmenarche	81.1% (30)	68.2% (30)	
Normal menarche			.253
Premenarche	18.9% (7)	31.8% (14)	
Normal menarche	81.1% (30)	65.9% (29)	
Early menarche	0% (0)	2.3% (1)	
Menstrual irregularity			.181
Premenarche	18.9% (7)	31.8% (14)	
No history of irregularity	10.8% (4)	18.2% (8)	
History of irregularity	70.3% (26)	50% (22)	
Age at start of irregularity			.100
Premenarche	18.9% (7)	31.8% (14)	
No history of irregularity	10.8% (4)	18.2% (8)	
Unknown	8.1% (3)	11.4% (5)	
9	0% (0)	2.3% (1)	
10	2.7% (1)	0% (0)	
11	21.6% (8)	4.6% (2)	
12	5.4% (2)	4.6% (2)	
13	5.4% (2)	9.1% (4)	
14	2.7% (1)	9.1% (4)	
15	10.9% (4)	2.3% (1)	
16	5.4% (2)	0% (0)	
17	5.4% (2)	2.3% (1)	
18	2.7% (1)	0% (0)	
20	0% (0)	4.6% (2)	
Symptoms of irregularity ^a			
Premenarche	18.9% (7)	31.8% (14)	.187
No irregularities	10.8% (4)	18.2% (8)	.352
Cycles <28-30 d	37.8% (14)	22.7% (10)	.138
Cycles >28-30 d	29.7% (11)	29.6% (13)	.986
Abnormally heavy bleeding	24.3% (9)	29.6% (13)	.599
Abnormally light bleeding	16.2% (6)	13.6% (6)	.745

	<u>Ovary sparing</u>	<u>Oophorectomy</u>	<i>P</i>
	n = 37	n = 44	
Painful menses	59.5% (22)	27.3% (12)	.003
No menses for <6 mo	10.8% (4)	6.8% (3)	.697
Unknown	2.7% (1)	0% (0)	.457
Achievement of menstrual regularity			.354
Premenarche	18.9% (7)	31.8% (14)	
No irregularities	10.8% (4)	18.2% (8)	
Achieved without hormonal regulation	16.2% (6)	6.8% (3)	
Achieved with hormonal regulation	21.6% (8)	13.6% (6)	
Not achieved	29.7% (11)	29.6% (13)	
Unknown	2.7% (1)	0% (0)	
Pregnancy			.078
Premenarche	18.9% (7)	31.8% (14)	
None and no attempts	81.1% (30)	59.1% (26)	
Successful with living children	0% (0)	6.8% (3)	
Attempts unsuccessful	0% (0)	2.3% (1)	
Hormonal suppression	32.4% (12)	29.6% (13)	.779
Onset of puberty			.544
Prepubertal	13.5% (5)	20.5% (9)	
Normal onset	5.7% (2)	61.4% (27)	
Precocious	10.8% (4)	15.9% (7)	
Delayed	0% (0)	2.3% (1)	
Symptoms of abnormal onset ^a			
Prepubertal	13.5% (5)	20.5% (9)	.411
Normal puberty	83.8% (31)	77.3% (34)	.463
Pubic hair	10.8% (4)	11.4% (5)	1.0
Breast development	8.1% (3)	13.6% (6)	.43
Axillary hair	2.7% (1)	4.6% (2)	1.0
No growth	0% (0)	2.3% (1)	1.0

^a A person may have more than 1 symptom. χ^2 Was used when all expected cell counts are more than 5. Fisher's Exact test was used when expected cell counts are less than 5. Wilcoxon rank sum test was used to compare continuous variables.