Imaging in gynecological disease: ultrasound features of malignant ovarian yolk sac tumors (endodermal sinus tumors)

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Running Headline: Ultrasound features of malignant yolk sac tumors (endodermal sinus tumors)

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CONTRIBUTION

What are the novel findings of this work?

There are no prior published papers on transvaginal sonographic features of yolk sac tumors.

What are the clinical implications of this work?

Sonographic features in combination with clinical information and tumour markers can aid in diagnosing yolk sac tumors. A correct diagnosis would make fertility sparing surgery a potential option.

ABSTRACT

Objectives

To describe the clinical and sonographic characteristics of malignant ovarian yolk sac tumors.

Methods

In this retrospective multicenter-study we included 21 patients with a histological diagnosis of ovarian yolk sac tumor where still images and/or videoclips were available. Ten patients collected from the IOTA-studies, had undergone a standardized preoperative ultrasound examination by an experienced ultrasound examiner between 1999 and 2016. The remaining eleven were identified through medical files, where images were retrieved from local image work stations and PACs systems. All tumors were described using IOTA terminology. The collected images and video clips were used for additional characterization by two observers.

Results

All cases were pure yolk sac tumors except for one, that was a mixed tumor (80% yolk sac tumor and 20% embryonic carcinoma). Median age at diagnosis was 25 (Interquartile range, IQR 19.5-30.5) years. Seventy-six percent (16/21) were FIGO stage I-II when diagnosed. 58% (11/19) women felt pain during examination and one patient presented with ovarian torsion. Median S-AFP level was 4755 µg/L (IQR, 1071 - 25303) and CA-125 126 kU/L (IQR, 35-227). On ultrasound assessment 95% (20/21) of tumors were unilateral. The median of the maximal tumor diameter was 157 mm (IQR 107-181), and the largest solid component 110 mm (IQR 66-159). Tumors were classified either as multilocular-solid (10/21, 48%), or as solid (11/21, 52%). Papillary projections were found in 10% (2/21) of the cases. Most tumors (20/21, 95%) were well vascularized (color score 3-4) and none of the tumors had acoustic shadowing. Malignancy was suspected in all cases except for the patient with torsion, which presented with a color score of 1 and was classified as probably benign. Image and video clip quality was considered as adequate in 18/21 cases.

Reviewing the images and videoclips we found that all tumors contained both solid components and cystic spaces, and that 89% (16/18) of the tumors had an irregular, still fine-textured, and slightly hyperechoic solid tissue, giving them a characteristic appearance.

Conclusion

Malignant ovarian yolk sac tumors are often detected at an early stage, in young women usually in the second or third decade of life, presenting with pain and markedly elevated S-AFP. On ultrasound yolk sac tumors are mostly unilateral, large, multilocular-solid or solid, with fine-textured slightly hyperechoic solid tissue, and rich vascularization.

AIM

Malignant yolk sac tumors are rare, and there is scarce data on their morphological appearance on ultrasound examination. The aim of this study was to describe gray-scale and color Doppler ultrasound features of malignant ovarian yolk sac tumors (endodermal sinus tumors), in order to facilitate their preoperative diagnosis, and to determine if these tumors have a specific appearance.

BACKGROUND

Epidemiology

Ovarian tumors are commonly classified as epithelial, non-epithelial or metastatic ovarian tumors from another primary malignancy. Germ cell tumors are a subgroup within the non-epithelial group and make up about 15-20% of all ovarian tumors[1]. The most common tumor of the germ cell tumors is the benign mature teratoma. Endodermal sinus tumors or, more commonly called yolk sac tumors, are also germ cell tumors but are rare and malignant. Other germ cell tumors are dysgerminoma, immature malignant teratoma, choriocarcinoma, and embryonal carcinoma. Yolk sac tumors derive from extraembryonic cell types and account for about 1% of all ovarian malignancies[2, 3]. Median age at presentation is 18-25 years[3], the tumor is rarely bilateral[4, 5], and most tumors present at an early stage[6]. Yolk sac tumors can occur and be treated during pregnancy[7, 8]. Yolk sac tumors may also appear in the testis and in extra gonadal locations[9]. Ovarian Yolk Sac Tumors appear both in pure forms or as part of a mixed germ cell tumor. In a recent case series, 51% (129/251) of yolk sac tumors presented in the pure form, the remaining being mixed[6]. By definition, mixed germ cell tumors consist of two or more types of malignant germ cell components[10]. The most common mixture is that of dysgerminoma and yolk sac tumor[11], other known associations are embryonal carcinoma, choriocarcinoma, or immature teratoma[9, 10]. Therefore, in the pathological report, these cases should be referred to as mixed

germ cell tumor, describing the extent and percentage of all the germ cell components observed[12].

Microscopic appearance

Histologically the yolk sac tumor is multifaceted with a diversity of features[9, 13-15]. The most characteristic histologic feature of yolk sac tumors is a reticular, glomerulus-like, structure[14], which caused Schiller to describe the tumor in 1939 as of Mesonephric origin. In 1959, Teilum revised this description and stated its extraembryonic germ cell origin[14, 16]. Teilum named the tumors "endodermal sinus tumors" because of their resemblance to endodermal sinuses in the rat placenta[9, 16]. Later the term "yolk sac tumors" were stated and both terms are still commonly used[1, 9, 13, 14]. The resemblance of yolk sac tumors to endodermal sinuses is due to the presence of sinuses, also called "Schiller-Duval bodies", that are composed of a central blood vessel lined by a layer of cuboidal or columnar cells[1, 3, 16]. The Schiller-Duval bodies are cross sections of papillary formations in a reticular labyrinth (Figure 1A and 1B). When present the 'Schiller-Duval bodies' are diagnostic of yolk sac tumors, but they are only found to be the predominant component in 20 % of cases[17]. When the reticular, glomerulus-like pattern merges, its interpapillary spaces can create a micro cystic appearance[9, 14]. Conspicuous intracellular and extracellular hyaline droplets are present in all tumors[1]. The histological diversity of the yolk sac tumors can sometimes make them difficult to diagnose, as they can mimic other tumors like hepatoid carcinoma and clear cell carcinoma[9, 15]. The first approach for diagnosing yolk sac tumors relies on the classical morphological parameters observed in haematoxylin and eosin stained sections (Reticular/ poly vesicular/ glandular/ hepatoid pattern/ Shiller-Duval bodies/ hyaline droplets)[9, 13, 14, 16]. Immunohistochemistry for α-feto protein (AFP) and glypican-3

represents a useful tool to confirm the morphological suspicion; however the final diagnosis mainly relies on the morphological features[14, 18].

Macroscopic appearance

Tumor size varies from 5 to 50 cm^3. The external surface of the tumor appears smooth and glistening with a cut surface that is tan to yellow/gray. Yolk sac tumors are mostly solid tumors with cystic components, and these cystic components span in diameter from a few millimeters to 2 cm[9, 16, 17] (Figure 2 and 3). Larger cystic degeneration is sometimes present, consisting of hemorrhage and necrosis[1].

Clinical symptoms

The most common symptom is abdominal pain followed by abdominal enlargement[3]. Duration of symptoms is often brief due to the rapid growth. About 10% of patients present with acute abdomen resulting from torsion, hemorrhage, or tumor rupture[19]. Other symptoms may be fever, abnormal vaginal bleeding or ascites[17]. Serum α-feto protein (S-AFP) is a useful marker, as elevated levels of S-AFP are present in almost 100% of cases, although elevated levels can also be present in other germ cell tumors[20, 21]. S-AFP levels can moreover be used to assess treatment effects and to detect a relapse[21-24]. Elevated S-CA-125 can also be present [20].

Prognosis

Though highly malignant, yolk sac tumors, are effectively treated with a combination of surgery and chemotherapy. Before the introduction of platinum therapy the prognosis was pessimistic with a 3-year survival of 13%[17]. Modern treatment schedules with BEP (bleomycin, etoposide, cisplatin)[4] improved the 5-year survival rate in yolk sac tumors to the current 94.8%, 97.1%, 70.9% and 51.6% for with FIGO (International Federation of Gynecology and Obstetrics) stage I, II, III, IV to, respectively[5]. Yolk sac tumors often present at an early stage and prognosis is favorable even in women with metastatic disease [6]. Fertility sparing surgery (i.e. unilateral salpingo-oophorectomy, omentectomy, peritoneal washings and biopsies) is found to be equally as effective as radical surgery. This is also true in women with metastatic disease[5, 25, 26] partly because it is rarely a bilateral disease[27]. Intraperitoneal seeding is the most common pattern of spread. Hematogenous spread is rare at time of diagnosis and distant metastasis is most commonly present as malignant pleural effusion and liver metastasis[28].

CASE SERIES

Materials and methods

We invited IOTA (International Ovarian Tumor Analysis) collaborators to take part in this retrospective survey. We extracted cases with a histological diagnosis of yolk sac tumor from the IOTA database and asked centers to contribute images and additional clinical and sonographic data. We also asked the IOTA collaborators to search their patient files and local image work stations and PACs systems for additional cases. Inclusion criteria were preoperative transvaginal ultrasound scan with detailed documentation in the form of archived images, video clips or a detailed ultrasound report. Eleven ultrasound centers contributed with 21 cases to the study (Bologna (n=3), Barcelona (n=3), Rome (n=3), Monza (n=3), Milan (National Cancer Institute) (n=1), Bangalore (n=2), Cagliari (n=1), Leuven (N=1), Navarra (N=1), Stockholm (n=2). Women from the IOTA studies had been examined between 1999 and 2016, and those investigated outside the IOTA protocol between 2007 and 2017.

All patients had been preoperatively examined with transvaginal ultrasound (supplemented with a transabdominal scan, if necessary), using a standardized examination technique [29]. All the ultrasound examiners had more than 10 years' experience in gynecological ultrasound, and the ultrasound examinations were carried out using high-end ultrasound equipment. The frequency of the vaginal probes varied between 5.0 and 9.0 MHz and that of the abdominal probes between 3.5 and 9.0 MHz.

For women prospectively included in the IOTA studies, clinical data and ultrasound characteristics were obtained from the IOTA databases. For women who had been examined outside the IOTA study protocol, and in case of missing information in the IOTA database, information and ultrasound images were retrospectively retrieved from the patients´ medical files and they were

entered into an Excel file by the principal investigator at each center. Final histology, tumor grade, and FIGO stage were registered. In addition, we asked the investigators to report tumor markers (CA-125, AFP and β-hCG) if analyzed, at the time of diagnosis. The masses were described using the terms and definitions published by the IOTA consortium [29]. The presence of ascites and fluid in the pouch of Douglas was noted. The vascularization of the tumors on color Doppler was described using the IOTA color score: no detectable blood flow (color score=1), minimal blood flow (color score=2), moderate blood flow (color score=3) or abundant blood flow (color score=4). In case of bilateral tumors only the largest tumor (if similar appearance) or the tumor with the most advanced pathology was included according to IOTA terms and definitions[29].The specific diagnosis suggested by the original ultrasound examiner in the IOTA database or in the original ultrasound report was recorded.

In addition to using the information collected in the IOTA database and in the patients' medical records, two examiners (EE and PA) with more than 20, and 7 years' experience in gynecological ultrasound re-assessed available ultrasound images and video clips (most of them electronic) of yolk sac tumors with the aim to identify ultrasound patterns typical of yolk sac tumors.

Statistical analysis

Continuous data are presented with means or median (interquartile range, IQR), and categorical data by frequencies and percentages. Analyses were performed using Microsoft Excel 2016 or IBM SPSS v.25 (International Business Machines Corp.; New Orchard Road; Armonk; New York 10504; 914-499-1900).

RESULTS

21 patients with histological diagnosis of ovarian yolk sac tumor were identified and included in this study. All cases were pure yolk sac tumors except for one (20/21), that was a mixed germ cell tumor consisting of 80% yolk sac tumor and 20% embryonic carcinoma. Ten patients (48%) were previously included in the IOTA studies, the remaining 11 were identified from local clinical and image databases. In one patient, there were no images or video clips available. In another two cases the images could not be confidently re-evaluated due to poor image quality, but we received clinical data and descriptive information of these tumors. Clinical characteristics are shown in Table 1. Median age was 25.0 years (IQR 19.5 – 30.5), 38% (8/21) were nulliparous, and 76% (16/21) had a FIGO stage I-II tumor. S-AFP levels were elevated in 95% of cases and S-CA-125 in 75%. β-hCG levels were measured in 11 cases and were not elevated in any of these cases.

Ultrasound characteristics are shown in Table 2. Ninety-five percent (20/21) of the tumors in this study were unilateral, one was bilateral. The largest tumor diameter was found to be in median 157 mm. All tumors were either classified as solid (10/21, 48%) or multilocular-solid (11/21, 52%) and 9,5% (2/21,) had papillary projections. Almost all tumors (20/21, 95%) were well vascularized (color score 3-4), Figure 4. Only the twisted yolk sac tumor (Case 11 in Table 3) had no detectable blood flow, this was also the only tumor that was preoperatively classified as probably benign. According to the ultrasound report 5/21 tumors were classified as probably malignant, and 15/21 were classified as certainly malignant. None of the lesions showed any acoustic shadowing. Only 38% (8/21) of the cases presented with ascites.

Eighteen cases were subjectively re-assessed by two observers using pattern recognition. In the remaining cases images were either missing (n=1), or of insufficient quality to allow for adequate assessment (n=2). The two observers evaluating the gray-scale and power Doppler ultrasound images in these 18 cases agreed in following description: Tumors were classified as either solid (Figure 2 and 5) or multilocular-solid (Figure 3 and 6). The majority (16/18, 89%) of tumors had

inhomogeneous but still fine-textured and slightly hyperechoic solid tissue, in solid tumors this giving rise to a 'Lunar-surface' appearance. The two cases with papillary projections are shown in Figure 7. The two cases without hyperechoic solid tissue were in the multilocular-solid group, having >10 locules (Figure 8). The multilocular-solid tumors resemble other multilocular-solid tumors, for example granulosa cell tumor with "swiss cheese" appearance. Although the appearances of these two tumor types do overlap, there might be discrete differences as the solid tissue in granulosa cell tumors has a coarser, slightly less echogenic, texture, and more numerous and irregular locules (Figure 9).

Detailed sonographic and demographic data for the included cases are found in Table 3. One woman had a personal history of serous borderline ovarian tumor.

DISCUSSION

In this study we describe the clinical and sonographic characteristics of malignant yolk sac tumors. Yolk sac tumors are often diagnosed at an early stage, in young women presenting with abdominal pain and markedly elevated S-AFP. On ultrasound tumors present as unilateral, large, well vascularized, multilocular-solid or solid lesions.

To our knowledge this is the first study describing the sonographic appearance of yolk sac tumors. A strength of our case series is that all but one, were pure yolk sac tumors making our sonographic findings representative of this particular histological entity. A limitation is the small sample size, the retrospective study design and the lack of optimal quality images for some cases. These facts may have limited the possibility to describe all variations and features of yolk sac tumors, also resulting in missing clinical information on S-AFP and S-CA-125 in some cases.

We have found no studies on transvaginal gray-scale ultrasound features of ovarian yolk sac tumors. One study of Levitin et al. from 1996[30] presented seven cases with abdominal scans. The description of those seven tumors is "both echogenic and hypoechogenic components with hypoechoic or anechoic elements predominating in four tumors". There is also a case report on an ovarian yolk sac tumor from Hung et al.[31] describing "a large mixed cystic and solid mass with a diameter of 19 cm occupying the pelvic cavity". Both these descriptions match with our findings. The ultrasound pattern of yolk sac tumors in this study appears to be consistent with the macroscopic gross appearance of textbooks and reviews cited above[1, 9, 16, 17] . This material had one case (5%) of bilateral tumors which matches earlier findings with larger samples showing bilateral disease 6 % of cases[5].

The demographic data, age and stage, of our series as compared to other studies lie in the higher age range of age (median 25 years vs. 18-25 years) as well as stage I-II (76% vs. 38-70%), respectively[2, 4, 5, 25, 32]. One explanation to the slightly higher median age in our series, could be

that yolk sac tumors often present in a pediatric population and are managed by pediatricians, where transvaginal ultrasound is usually not performed, although trans-rectal ultrasound could be a valuable option. The highly elevated S-AFP levels and S-CA-125 levels (median S-CA-125 concentration of 126 U/mL) are in agreement with other studies[3, 4].The majority of patients reported pain during examination. Pain is probably caused either by the rapid growth of the tumor leading to necrosis, or by the large tumor size. Moreover, 10% of patients with malignant germ cell tumors present with an acute abdomen resulting from torsion, hemorrhage, or tumor rupture[19]. In our series, one case presented with clinical symptoms of torsion and it was the only case classified preoperatively as probably benign, maybe because no blood flow could be detected. This highlights the fact that both doppler and grayscale ultrasound morphology may be altered and difficult to assess in torted lesions[33].

We know that yolk sac tumors can be present together with other benign or malignant germ cell tumor in a mixed form around 50% of cases[6, 9, 34]. In supplementary Video 1 we show a case of a germ cell tumor including both yolk sac and benign dermoid (hyperechoic with shadowing) components. Ultrasound features of pure yolk sac tumors might not be superimposable to ultrasound features of mixed yolk sac tumors.

Although the sonographic characteristics of yolk sac tumors and granulosa cell tumors overlap there might be some subtle differences in the echogenicity of solid tissue (Figure 9), as the solid tissue in yolk sac tumors may appear fine-textured, slightly hyperechoic appearance and that of granulosa cell tumors more granulated. Still differential diagnosis can be difficult as both tumor types can be bulky, solid or multilocular-solid, with abundant vascularization [35]. However, combining sonographic features with clinical information (age, symptoms), and tumor markers might provide a clue to the most probable diagnosis. The sonographic appearance can guide which tumor markers that should be assessed to discriminate non-epithelial from epithelial tumors as well as giving a hint on the specific diagnosis, for example dysgerminomas may present

with elevated LDH and hCG[36], granulosa cell tumors with elevated estrogen and inhibin[35], Sertoli-Leydig cell tumors with elevated testosterone or andostendione[37], immature teratoma with AFP and CA 19-9[20], and yolk sac tumor with AFP. We believe that an increased knowledge on the sonographic appearance of rare tumors may improve clinical decision making and patient counseling for example discussing the possibility of fertility-sparing surgery, and the need of postoperative chemotherapy. Further collaboration through international multicenter studies including image databases can facilitate a growing knowledge on this topic.

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LEGENDS

Figure 1A. Reticular pattern with multiple Schiller-Duval bodies, **overview**

Figure 1B. **Close up of** a Schiller-Duval body.

Figure 2. Gross appearance of a yolk sac tumor, solid

Figure 3. Gross appearance of a yolk sac tumor, multilocular-solid

Figure 4. Typical Color Doppler findings in **four different** yolk sac tumor**s**

Figure 5. Ultrasound images of **six different** solid yolk sac tumors

Figure 6. Ultrasound images of **six different** multilocular-solid yolk sac tumors

Figure 7. Two yolk sac tumors with papillary projections

Figure 8. Two yolk sac tumors without hyperechoic solid tissue.

Figure 9. Comparing sonographic features of yolk sac tumors and adult granulosa cell tumors.

Supplementary Video: Yolk sac tumor and benign dermoid. The dermoid component is seen as the hyperechoic components with shadowing.

Table 1. Clinical characteristics

Age, years	25.0 (19.5-30.5)
Nullipara	16 (76)
Personal history of ovarian cancer	1 (5)
FIGO stage	
1	14
2	2
3	4
4	1
S-CA125* (normal <35 kU/L)	126 (35-227)
S-AFP** (normal <8 µg/L)	4755 (1071 - 25303)

Data presented as n (%) or median (interquartile range)

* = data missing in 1 case, ** = data missing in 5 cases

Table 2. Ultrasound Characteristics

Primary examination	
Pain during examination*	11 (58%)
Largest tumor diameter (mm)	157 (107-181)
Maximum diameter, solid component (mm)	110 (66-159)
Unilateral	20 (95%)
Tumortype	
multilocular-solid	10 (48%)
solid	11 (52%)
Number of locules	
≥ 10	5 (24%)
5 to 9	5 (24%)
0	11 (52%)
Papillary projections	2 (10%)
Irregular lesion	19 (90%)
Echogenicity of fluid	
anechoic	5 (24%)
low-level	6 (29%)
hemorragic	2 (10%)
mixed	4 (19%)
no fluid	4 (19%)
Colour score	
1	1 (5%)
2	0 (0%)
3	10 (48%)
4	10 (48%)
Ovarian crescent sign	1 (5%)
Shadowing	0 (0%)
Ascites	8 (38%)
Metastasis seen	3 (14%)
Diagnosis suggested by ultrasound examiner:	
Certainly benign	0 (0%)
Probably benign	1 (5%)
Uncertain	0 (0%)
Probably malignant	5 (24%)

Certainly malignant	15 (71%)
Review of images and video clips **	
Hyperechoic solid tissue	
yes	16 (89%)
no	2 (11%)

Data presented as median (interquartile range) or n (%)

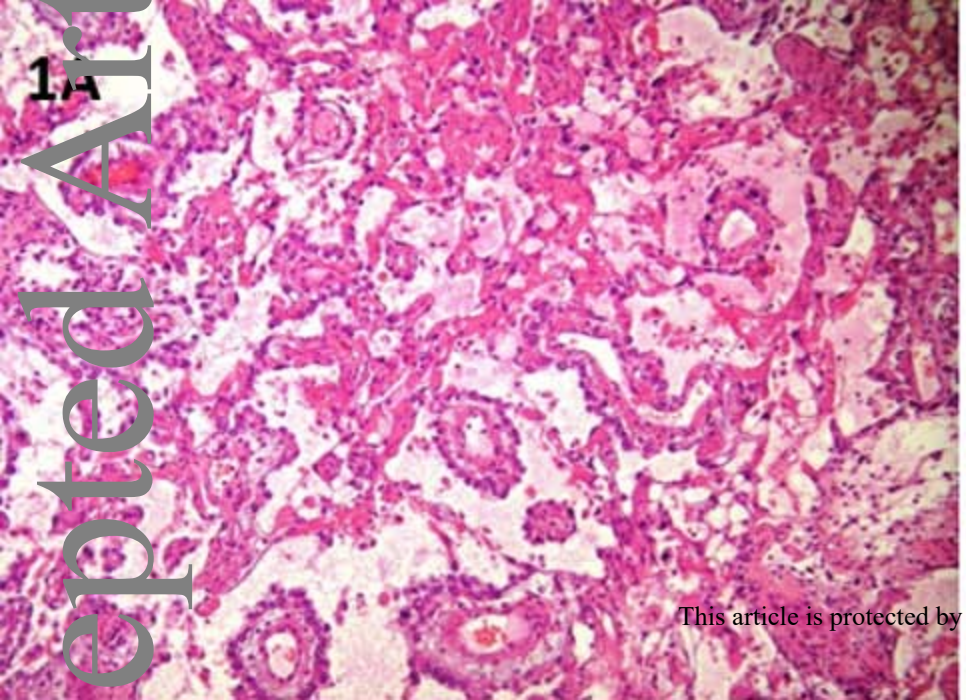
* Data on pain during examination available from 19 women

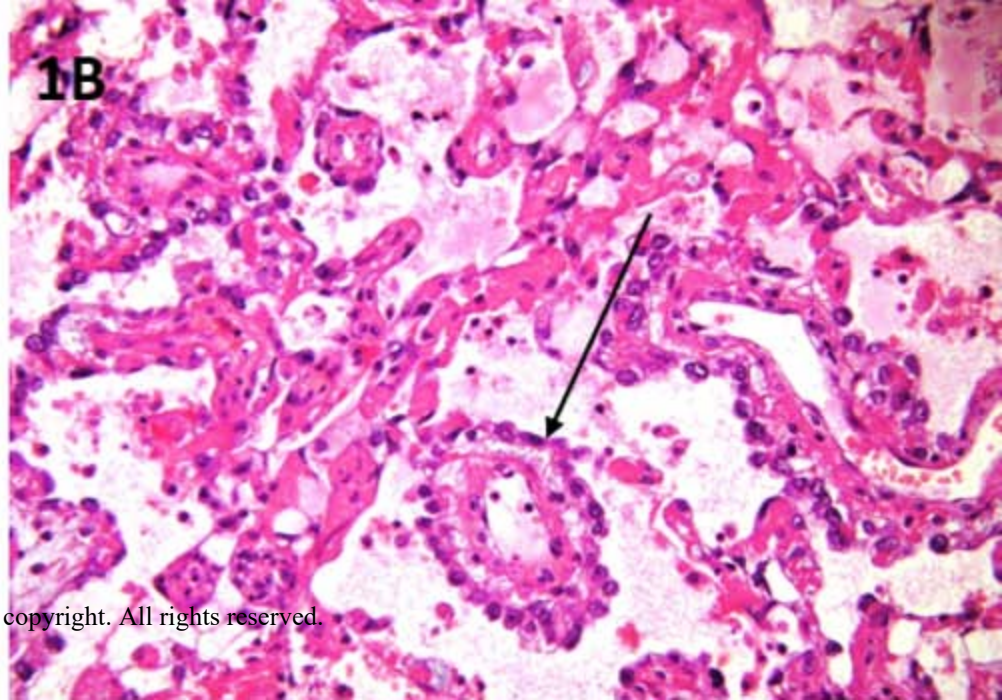
** Adequate images available from 18 tumors, 17 pure yolk sac tumors and one mixed germ cell tumor (80% yolk sac tumor 20% embryonal carcinoma).

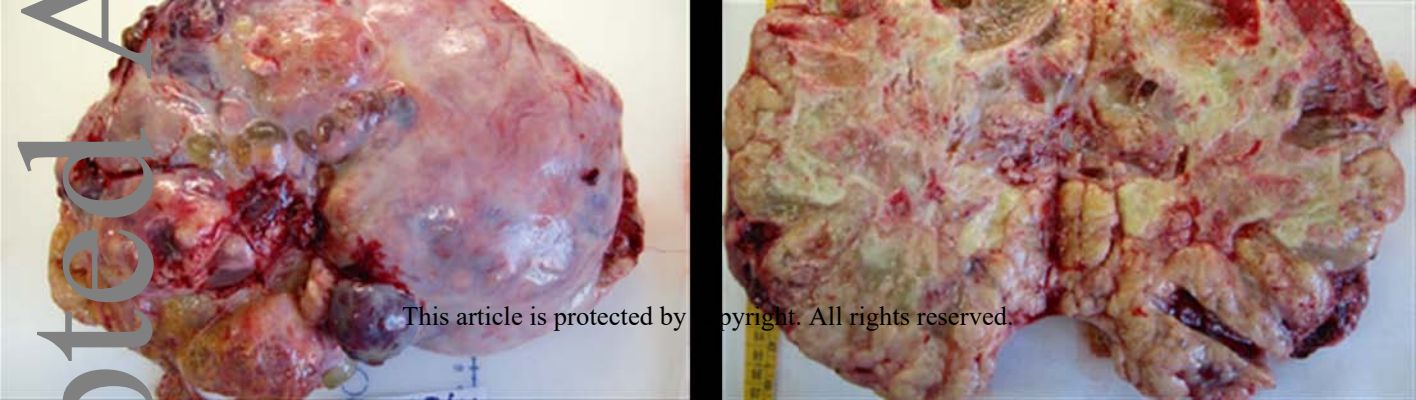
Table 3. Detailed demographic and sonographic characteristsics													
Case nr	Age	Parity	FIGO stage	CA125	AFP	Largest tumor diameter (mm)	Maximum diameter solid component (mm)	Tumor type	Number of locules	Hyperechoic solid tissue	Colour score	Ascites	Presumed histological diagnosis
1	20	0	1C	64	12200	159	159	solid	0	yes	4	no	malignant rare tumour
2	19	0	3B	350	50	80	80	solid	0	poor images	4	yes	malignant rare tumour
3	40	2	2B	201	2	50	30	multilocular-solid	>10	yes	3	yes	malignant rare tumour
4	25	1	4	625	NA	20	20	solid	0	poor images	3	yes	borderline tumour
5	25	0	1C	62	17	170	170	solid	0	yes	3	no	malignant rare tumour
6*	27	0	1B	170	NA	130	130	solid	0	yes	4	no	pirmary ovarian cancer
7	32	0	1A	74	1071	209	209	solid	0	yes	3	yes	pirmary ovarian cancer
8	11	0	1A	14	5651	62	58	solid	0	yes	3	no	pirmary ovarian cancer
9	37	0	3C	237	175600	120	114	solid	0	yes	3	yes	pirmary ovarian cancer
10	20	0	1A	77	4755	120	74	multilocular-solid	6	yes	4	no	malignant rare tumour
11	28	1	1	8	NA	110	52	multilocular-solid	5	no images	1	no	benign rare tumour
12	15	0	1	18	25303	180	98	multilocular-solid	9	yes	4	no	pirmary ovarian cancer
13	34	0	1A	24	53779	104	103	multilocular-solid	>10	yes	4	no	malignant rare tumour
14	21	0	1A	117	NA	157	149	multilocular-solid	8	yes	4	no	malignant rare tumour
15	28	0	3C	196	10874	159	159	solid	0	yes	4	yes	malignant rare tumour
16	30	3	1C	162	4346	211	119	multilocular-solid	>10	no	3	no	malignant rare tumour
17	23	0	2	135	3637	109	109	multilocular-solid	5	yes	3	yes	malignant rare tumour
18	31	1	1A	26	NA	330	110	multilocular-solid	>10	yes	3	no	malignant rare tumour
19	14	0	1	NA	NA	350	350	solid	0	yes	3	no	malignant rare tumour
20	26	0	1B	476	2435	181	38	multilocular-solid	>10	no	4	yes	pirmary ovarian cancer
21	19	0	3C	235	112000	181	181	solid	0	yes	4	no	malignant rare tumour

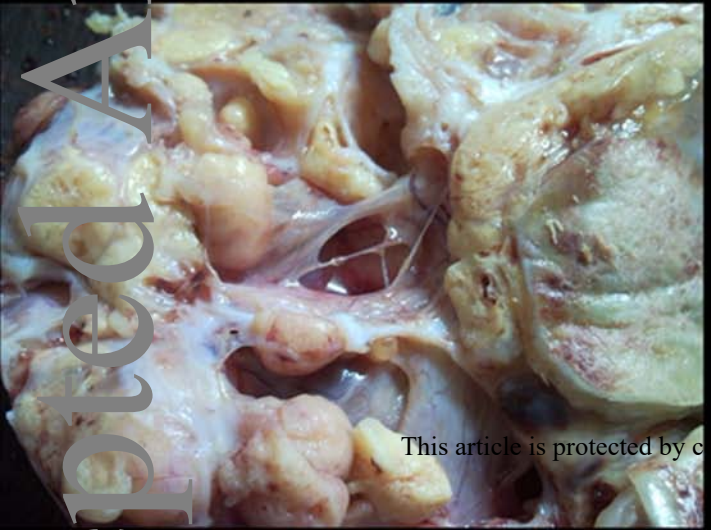
NA = data not avaliable

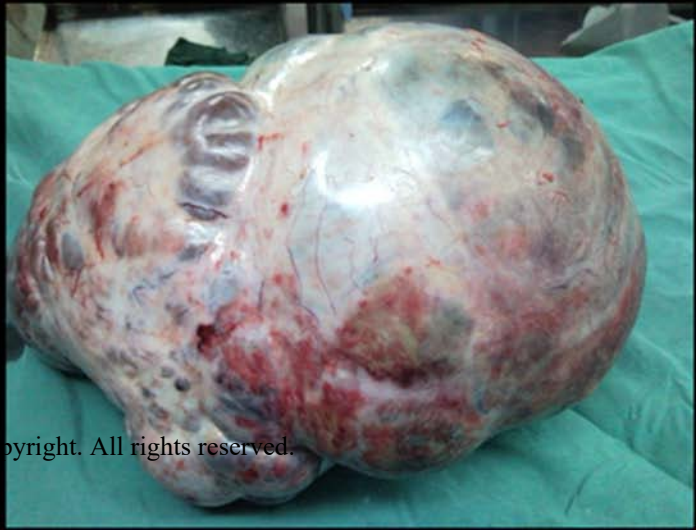
*Mixed germ cell tumor

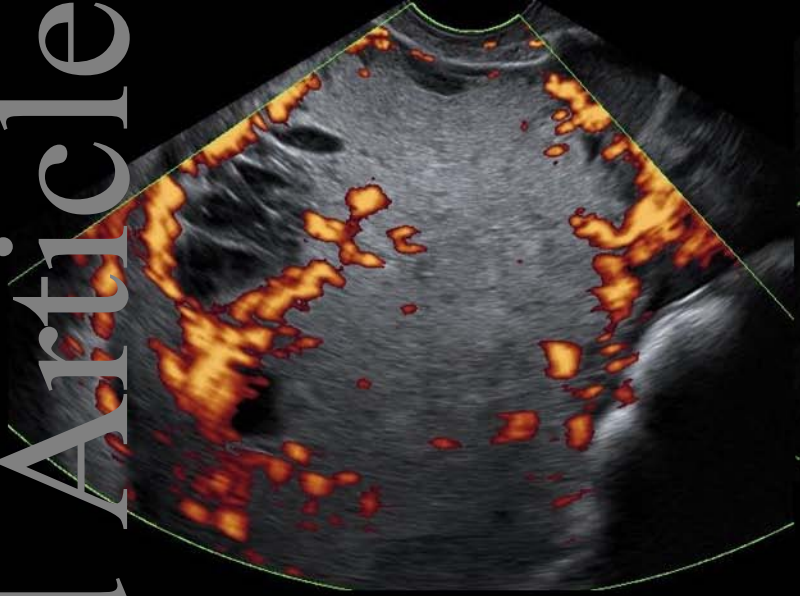


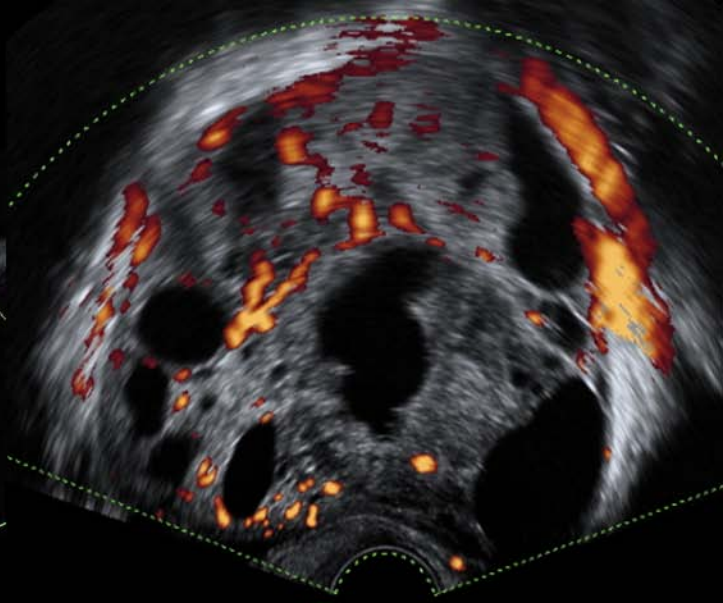


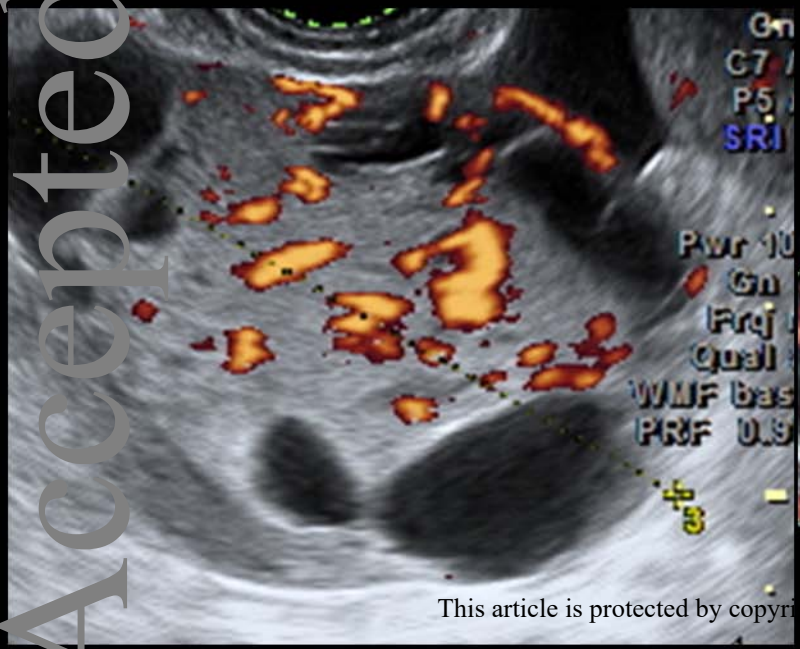


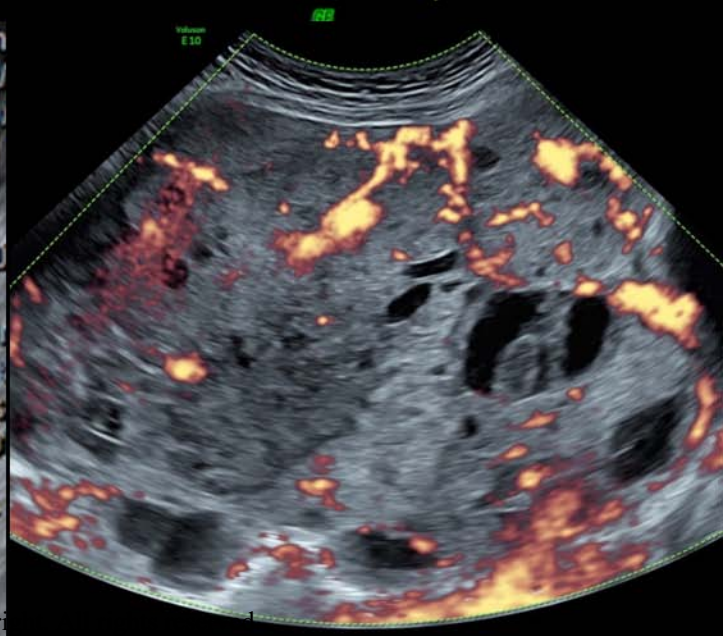


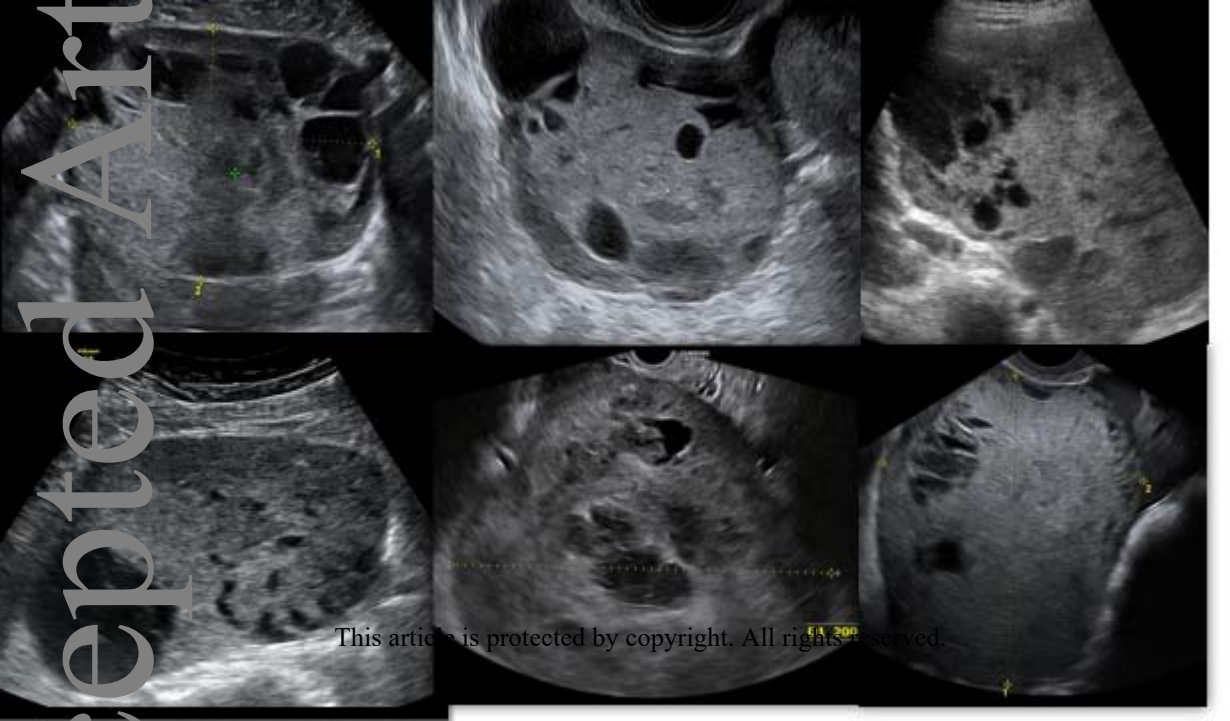


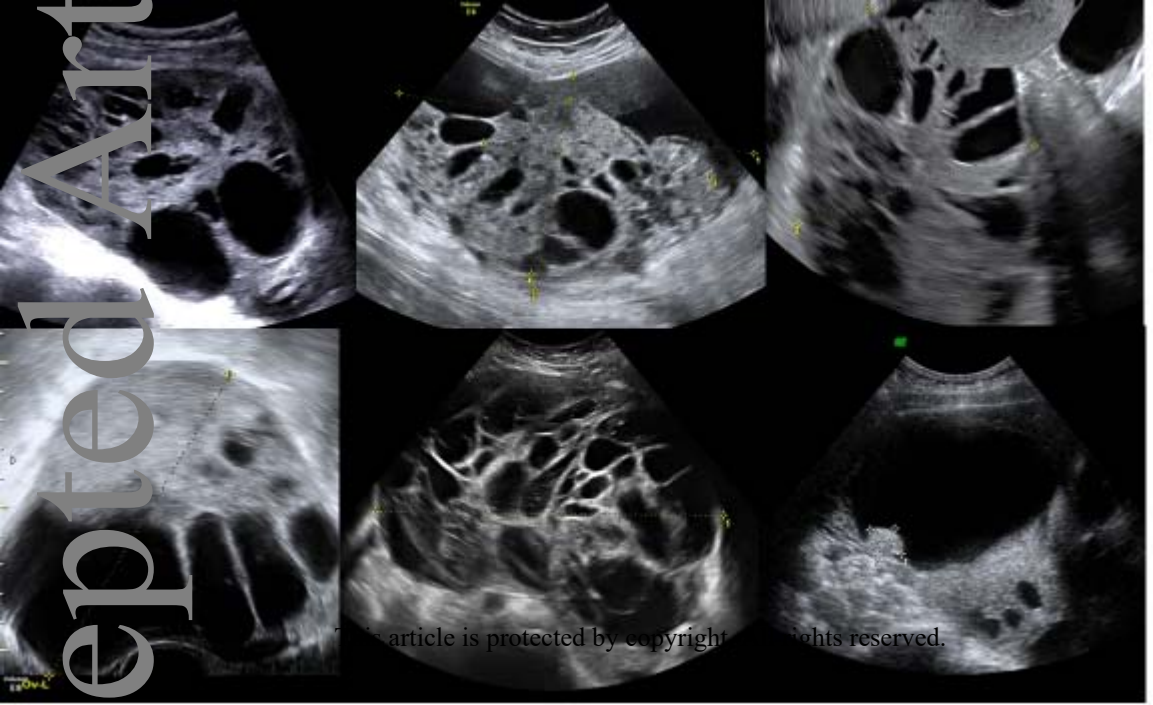




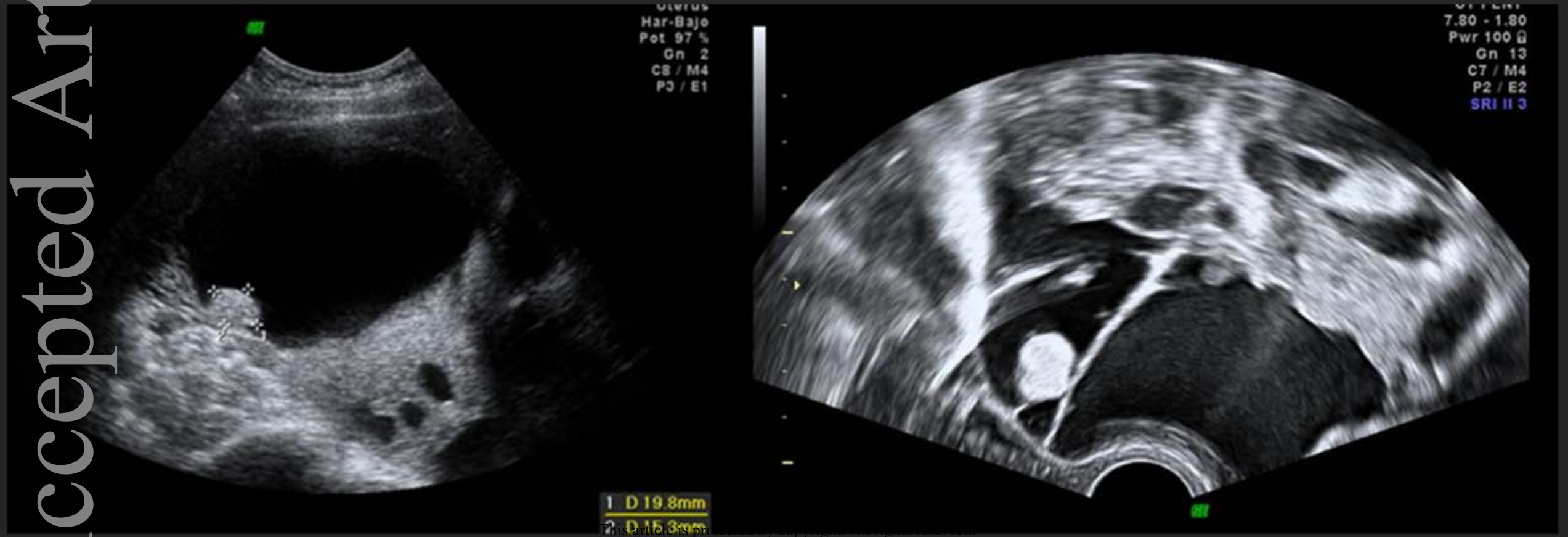




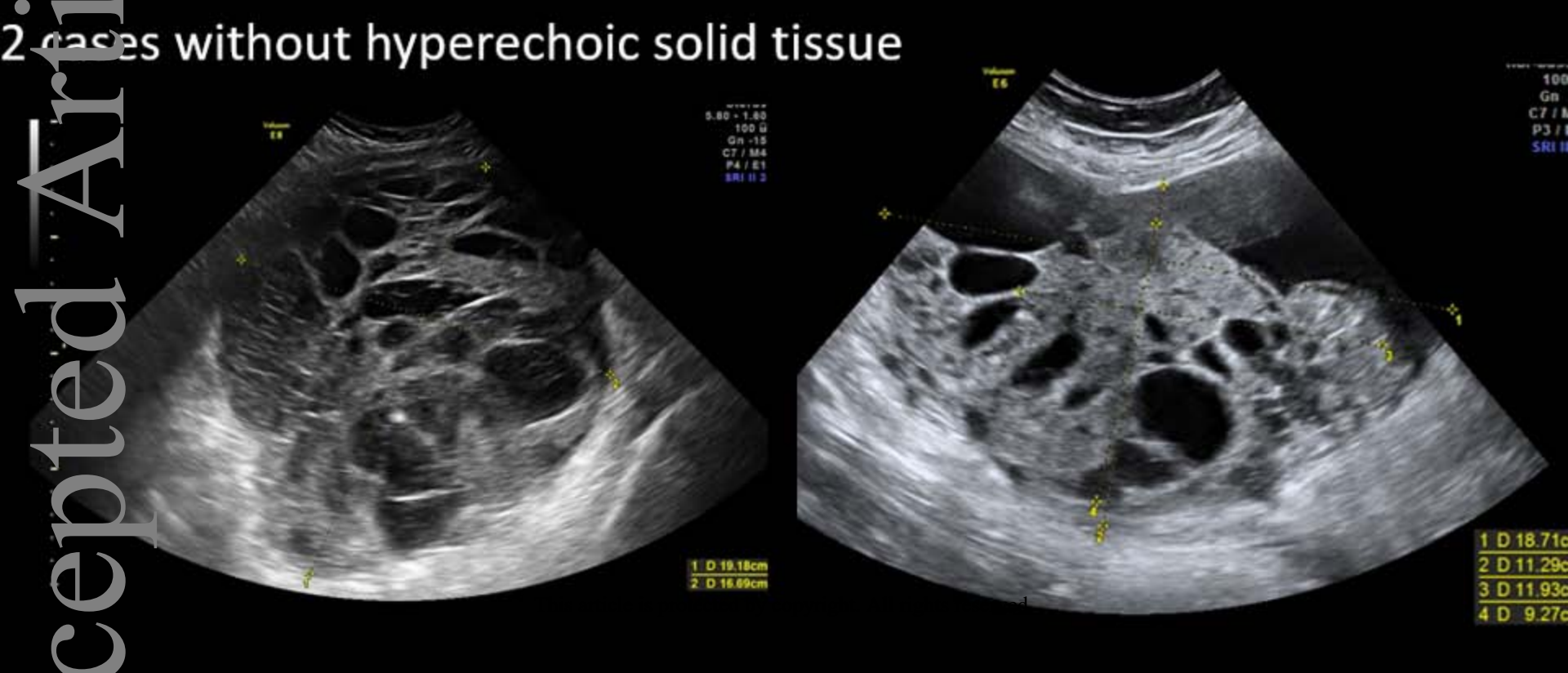




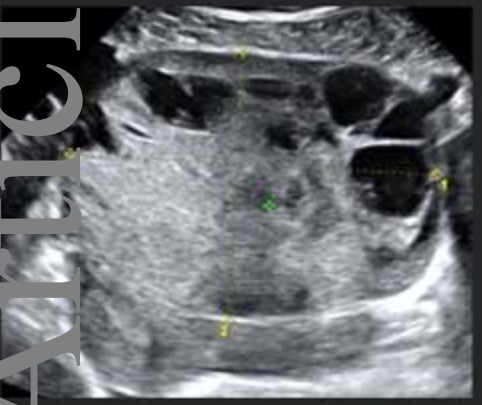
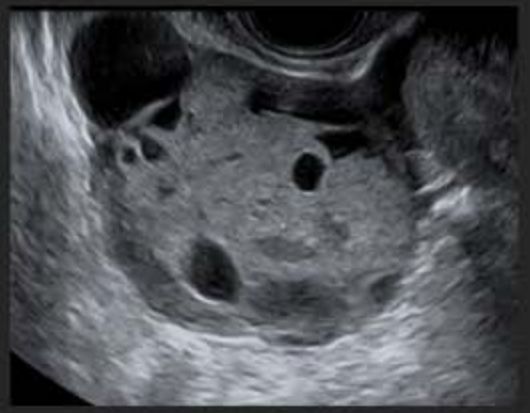
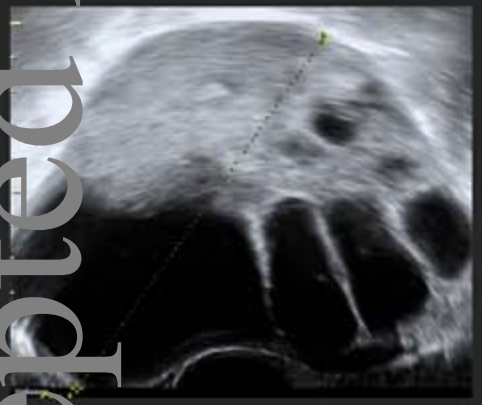
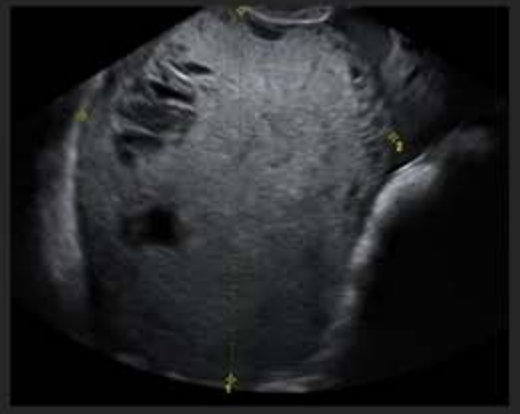
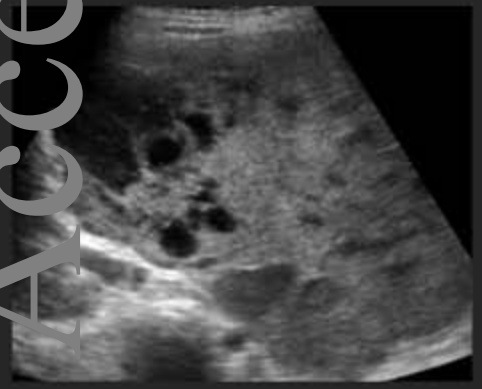
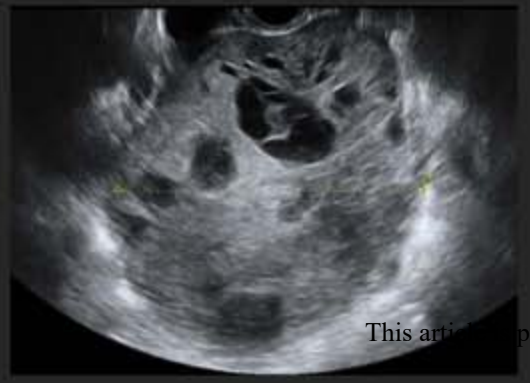
Two yolk sac tumors with papillary projections



2 cases without hyperechoic solid tissue



YOLK SAC TUMOR

GRANULOSA CELL TUMOR