Review

Discovery Medicine 2024; 36(181): 234–247
https://doi.org/10.24976/Discov.Med.202436181.22

Review on Sertoli-Leydig Cell Tumours of the Ovary

Corinne Muscat[1,*], Jean Calleja-Agius[1]

[1]Department of Anatomy, Faculty of Medicine and Surgery, University of Malta, Birkirkara Bypass, Msida MSD2080, Malta
*Correspondence: corinne.muscat.21@um.edu.mt (Corinne Muscat)
Published: 20 February 2024

Sertoli-Leydig cell tumours (SLCTs) represent a subset of mixed sex cord-stromal tumours (SCSTs), a rare form of non-epithelial ovarian tumours comprising less than 7% of malignant cases. Among other types of SCSTs, SLCTs are one of the more prevalent types observed in young adults. SLCTs are classified into 5 histologic categories based on differentiation levels and histological variants. Diverse chromosomal and genetic mutations have been identified in SLCTs, with the most well-studied being the genetic mutations observed in the Dicer 1, Ribonuclease III (*DICER1)* and the Forkhead Box L2 (*FOXL2)* genes. These mutations have important clinical implications and their mechanisms are discussed. Particularly, this review emphasizes the correlation between tumour differentiation, mutation status and virilization. Current common methods and difficulties in the clinical diagnosis of SLCTs are also considered, and the usefulness of immunohistochemistry is highlighted. Patient stratification for treatment is done according to the patient's age, stage of disease and prognostic factors. The gold standard of treatment is surgical resection and adjuvant chemotherapy is administered based on the risk of recurrence. The management of recurrence remains a major challenge. Apart from recurrence, there is also a risk of the development of a metachronous tumour, especially in patients with *DICER1* syndrome. Hence, the diagnosis of a SLCT has important implications for genetic testing and patient surveillance even if the management of the tumour is successful. This scoping review serves to consolidate current knowledge on SLCTs and advocates for future research advancements to refine diagnosis, management, and prognosis.

Keywords: Sertoli-Leydig cell tumours; sex-cord stromal; differentiation; *DICER1*; rare tumour

Introduction

Ovarian Sertoli-Leydig cell tumours are a type of sex cord-stromal tumour (SCST). Sex cords are structures that arise from embryonic ridges. In females, sex cords develop into cortical cords which give rise to ovarian follicles after further development, while in males, they give rise to the testis cords which develop into the rete testis. Stromal cells form the connective tissue of the ovary. Ovarian stroma has some differences from typical connective tissue, including a whorled appearance, high vascularity and an ability to gain endocrine function. Apart from the spindle-shaped cells typical of stromal tissue, there may also be cells such as Leydig cells, smooth muscle cells, decidual cells, luteinised stromal cells, and neuroendocrine cells [1].

A SCST is a tumour which develops from the uncontrollable division of either sex cord cells, stromal cells, or both [2]. These sex cord and/or stromal cells could differentiate into ovarian cell types, testicular cell types, and/or indifferent elements [3,4] (Fig. 1).

As shown in Fig. 2, Sertoli-Leydig cell tumours (SLCTs) of the ovary are classified under mixed sex cord-stromal tumours [5]. This is because, as their name suggests, they consist of both Sertoli and Leydig cells. Therefore, SLCTs originate from ovarian cells which differentiate into both testicular type sex cord cells and testicular type stromal cells. A pure Sertoli cell tumour of the ovary is classified as a sex cord tumour. Conversely, if only Leydig cells were involved, the tumour would be classified as a 'Leydig stromal tumour'.

The first case of a SLCT was reported by Pick in 1905, but it was misdiagnosed as an 'adenoma tubulare testiculare ovarii', a malformation within the ovotestis. Blair and Bell documented the first functioning tumour with virilizing features in 1915 [6]. However, a proper diagnosis of SLCTs could not be made until these tumours were adequately defined by Dr. Robert Meyer in 1931 [7]. Meyer (1931) [7] also subdivided SLCTs into 3 histological categories based on the extent of their differentiation: well-differentiated, intermediately-differentiated and poorly differentiated [8]. The most recent WHO classification includes the retiform variant of the tumour as a fourth subtype due to significant clinical and pathological differences [9]. However, there can be significant overlap between the retiform subtype and the intermediately or the poorly differentiated subtypes, because the latter degrees of differentiation often exhibit a retiform pattern.

Overall, SLCTs have received limited research attention despite being under review for a relatively long period. This paper offers a distinctive contribution to the existing literature on SLCTs as it provides a comprehensive overview of the topic, highlighting various key aspects

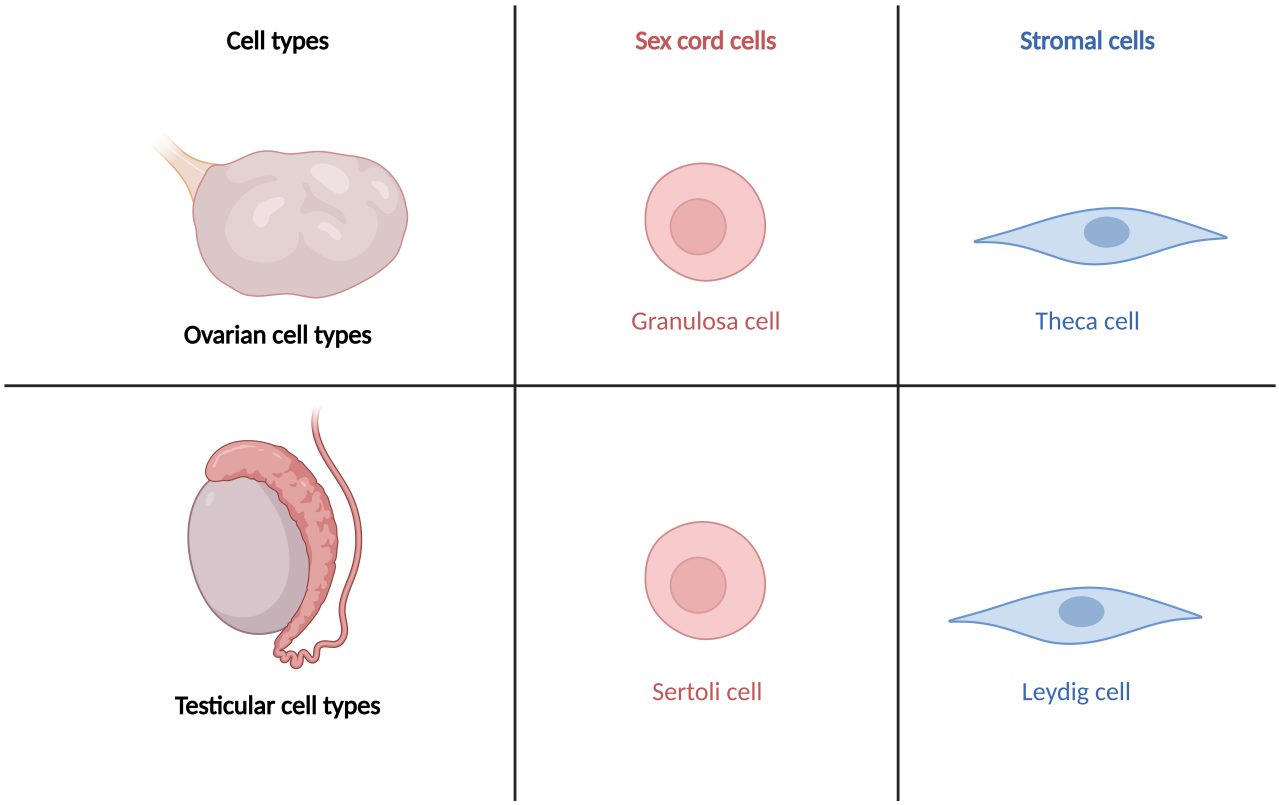

Fig. 1. The ovarian type and the testicular type sex cord and stromal cells. Created with BioRender.com (2023 BioRender, Science Suite Inc, Toronto, Ontario, Canada).

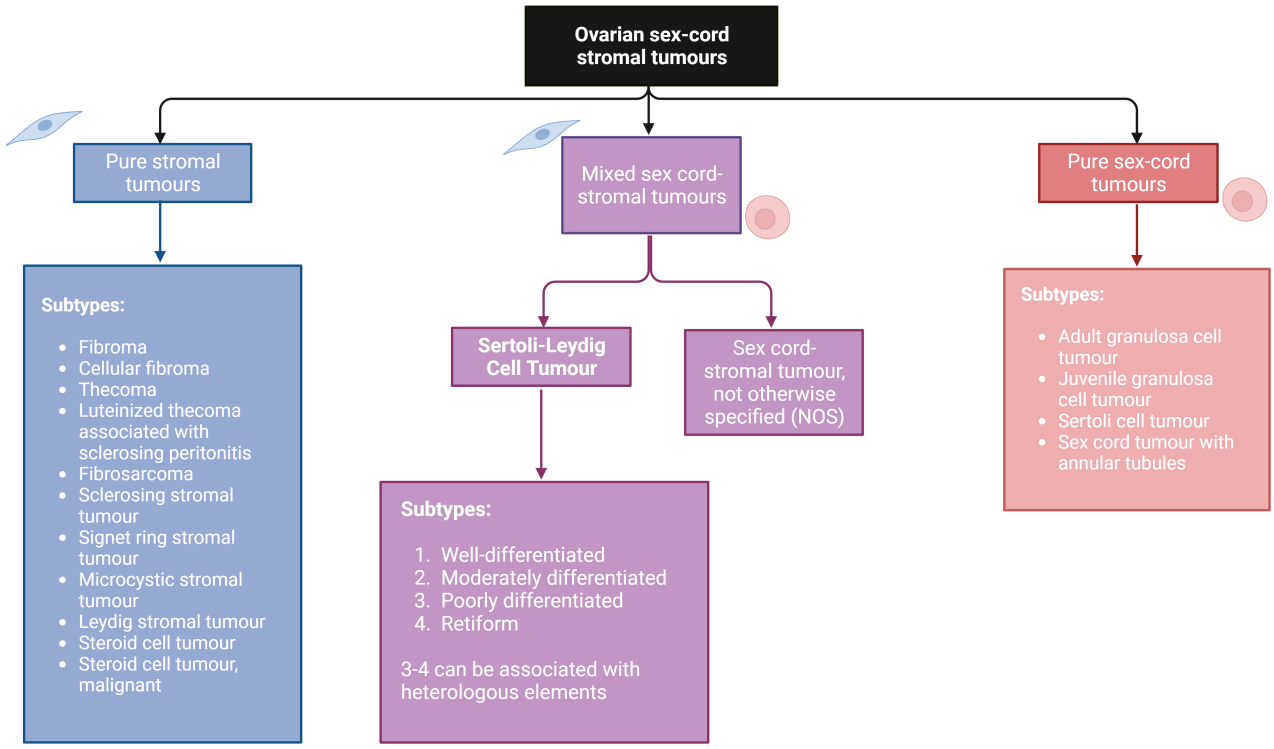

Fig. 2. World Health Organisation (WHO) classification scheme for ovarian sex cord-stromal tumours, 2014. Adapted from Al Harbi *et al.* (2021) [5], created with BioRender.com (2023 BioRender, Science Suite Inc, Toronto, Ontario, Canada).

Table 1. The prevalence of different histological types of SLCT [12].

Subtype	Percentage
Well-differentiated/Meyer type 1	11% of SLCTs
Intermediate type/Meyer type 2	54% of SLCTs
Poorly differentiated/Meyer type 3	13% of SLCTs
Retiform type	15% of moderately or poorly differentiated tumours
SLCT with heterologous elements	22% of moderately or poorly differentiated tumours

SLCT, Sertoli-Leydig cell tumour.

and novelties. These include an emphasis on genetic mutations in the Dicer 1, Ribonuclease III (*DICER1*) and Forkhead Box L2 (*FOXL2*) genes, potentially contributing to a deeper understanding of the molecular underpinnings of SLCTs. The paper also focuses on the clinical presentation of SLCTs, particularly the correlation between the degree of differentiation, the mutation status and the occurrence of virilization. It also highlights current common methods and difficulties in clinical diagnosis. Additionally, it discusses prognostic indicators such as stage, degree of differentiation, tumour size, and the presence of specific histologic elements, offering insights into treatment strategies, including the importance of postoperative chemotherapy for patients with a poor prognosis. Finally, the paper outlines future research directions aimed at improving the diagnosis, management, and prognosis of patients, thereby distinguishing itself as a forward-looking resource in the field.

Methodology

Literature reviews, case reports, original research and medical textbooks were used to compile information for this scoping review, with a total of 76 sources cited. Databases used to find relevant peer-reviewed papers include PubMed, Google Scholar and the university library databases. Only papers written in the English language were reviewed. The publishing dates of the sources used range from 1931 to 2023.

Epidemiology

SCSTs are a rare type of non-epithelial ovarian tumours which constitute less than 7% of malignant ovarian tumours [10], making them the fifth most common ovarian malignancy [11]. In their clinicopathological analysis of 207 cases, Young *et al*. (1985) [12] found that SLCTs are a subtype which makes up 0.5% of all ovarian neoplasms. SLCTs are one of the most common SCSTs in young adults, along with juvenile granulosa cell tumours. Although SLCTs have been reported in any age period, 75% occur in women between the ages of 20 and 39 [13]. Less than 10% of SLCTs are found in premenarchal or postmenopausal women [14]. As a result, the average age of SLCT patients is 24 years [15]. There are ethnic differences where SLCTs seem to be more common among African women [10].

The proportion of the different subtypes of SLCTs varies, and the degree of differentiation is related to age. Moderately differentiated SLCTs are the most common overall. Young *et al*. (1985) [12] found that most well-differentiated tumours occur at a mean age of 35 years, which is approximately a decade later than the mean age for the occurrence of moderately or poorly differentiated tumours. Additionally, moderately or poorly differentiated tumours with a retiform pattern or an underlying *DICER1* mutation tend to be found in even younger patients [3,16]. The retiform pattern is found in 15% of moderately and poorly differentiated tumours overall. Table 1 (Ref. [12]) summarizes the prevalence of each SLCT subtype, as well as the prevalence of SLCTs with heterologous elements. Although the retiform variant mostly occurs in the younger age group with an average age of 16 years [15], Rathi *et al*. (2015) [15] and Nwogu *et al*. (2017) [17] separately each reported a rare case of bilateral retiform variant of an SLCT in two different middle-aged women.

Guo *et al*. (2020) [18] conducted an analysis of 13 cases of SLCTs who were managed in one hospital in Shanghai between 2010 and 2019. They reported that the tumours had the following degrees of differentiation: 54% moderately differentiated and 46% poorly differentiated [18]. These results differ considerably from those of Young *et al*. (1985) [12] and various other studies which supported their results in the pelvis [19–22]. Guo *et al*. (2020) [18] pointed out that the proportion of well-differentiated tumours tends to be lower in cases from China, hence it is possible that these differences are due to ethnic and/or genetic variations.

Tumour Composition

Macroscopic Features

SLCTs are usually unilateral, with Young *et al*. (1985) [12] reporting bilateral involvement in only 1.5% of cases. Additionally, well-differentiated tumours have an average diameter of 5 cm, whilst the mean diameter of moderately and poorly differentiated tumours is 15 cm [15]. However, SLCTs may grow up to 35 cm [23].

Components of SLCTs may be entirely solid, entirely cystic, or mixed. Mixed SLCTs are the most common, making up 60% of SLCTs [13]. Most are predominantly solid with cystic areas [24]. The solid portions of SLCTs are usu-

Table 2. A comparison of the histological and cytological features of the different Meyer types [9,26–30].

SLCT Type	Meyer type I	Meyer type II	Meyer type III
Degree of differentiation	Well-differentiated	Moderately differentiated	Poorly differentiated or undifferentiated
Grade	1	2	3
Appearance	Well-circumcised tumour.	Identifiable lobular arrangement.	Sarcomatoid type tumour.
Arrangement of Sertoli cells within the stroma	Sertoli cells are arranged in solid or hollow tubules within a fibrous stroma containing clusters of Leydig cells.	Cellular areas of Sertoli cells are typically separated by oedematous stroma, or occasionally by fibrous stroma.	Widespread or spindle-shaped proliferation of Sertoli cells within a pleomorphic stroma.
Maturity of Sertoli cells	Mature Sertoli cells which lack atypia or mitotic figures.	Sertoli cells are immature, appear hyperchromatic and have a high nuclear to cytoplasmic ratio (indicative of rapid mitotic activity).	Sertoli cells are immature, appear hyperchromatic and have a high nuclear to cytoplasmic ratio (indicative of rapid mitotic activity).
Arrangement of Leydig cells	Leydig cells in singles, clusters or cords.	Leydig cells in clusters or singles, within the oedematous stroma or less frequently surrounding the lobules of Sertoli cells.	Foci of Leydig cells, typically unidentifiable.
Cytologic atypia and mitotic figures	Rare.	Rarely in both cell types.	Common.
Retiform patterns or heterologous elements	Absent.	May be present (typically endodermal elements).	May be present (typically mesenchymal elements).

ally fleshy, lobulated and frequently yellow [23]. Ovarian tumours which are golden yellow to orange may suggest that it is a steroid-producing tumour. Retiform variants or those SLCTs with heterologous elements often have cystic components. Haemorrhage and necrosis are features which belong to poorly differentiated tumours [9].

Microscopic Features

SLCTs are divided into five main histologic categories depending on their appearance under the microscope: well-differentiated; intermediate differentiated; poorly differentiated; retiform variant; and tumours with heterologous elements.

Microscopically, a well-differentiated tumour appears similar to a pure Sertoli cell tumour, but it also contains Leydig cells and stromal elements [25]. A moderately differentiated SLCT is characterized by anastomosing cords and trabeculae of columnar cells, and diagnosis relies on the detection of these features [26]. In a poorly differentiated tumour, the ovarian stroma is primitive and pleomorphic. The tumour might have foci resembling epithelial, mesenchymal or even germ cell tumours. Poorly-differentiated tumours are sarcomatoid type, meaning that at first glance under low-power or medium-power, they might be mistaken for a sarcoma, especially if intermediate-differentiated areas are not seen [27]. Hence, diagnosis of a poorly differentiated SLCT can prove to be difficult and a meticulous inspection of the tumour for cords of Sertoli cells and Leydig cells is necessary [24]. Table 2 (Ref. [9,26–30]) compares the histological and cytological features of the different Meyer types of SLCTs. Occasionally, there can be varying degrees of differentiation within the same tumour [26]. Additionally, there are some differences in presentation when SLCTs occur during pregnancy. Considerable areas of the tumour often have a distorted architecture due to significant intercellular oedema which may affect the diagnosis. In some pregnant patients, Leydig cells also aggregate to form large sheets [27].

Retiform foci are areas with structural and cytological characteristics that resemble the rete testis [15]. Tumours with a retiform pattern tend to be larger and more cystic. The retiform pattern consists of slit-like spaces, clefts and papillae lined with cuboidal to columnar cells [31]. Oedematous or gelatinous papillae or polyps often project into tubules and cysts [27]. Papillae are blunt with a hyalinized eosinophilic core or an oedematous core [15]. In contrast, polyps are sizeable polypoid projections into cysts [32]. Guo *et al*. (2020) [18] stated that in their analysis of 13 cases, a considerably larger number of tumours with a retiform pattern was observed in those patients without endocrine function when compared with the endocrine function group.

Heterologous elements within an ovarian tumour are those cell types which are not normally present in the ovary. Heterologous elements of different types continue to add to the wide variety of appearances which SLCTs can take. Meyer (1931) [7] also contributed to the idea of heterogeneity within SLCTs by recognizing heterologous mucinous epithelium, which is usually benign but it can exhibit cytologic atypia similar to a borderline or malignant tumour [27]. The mucinous epithelium is the most common heterogenous element in SLCTs, followed by carci-

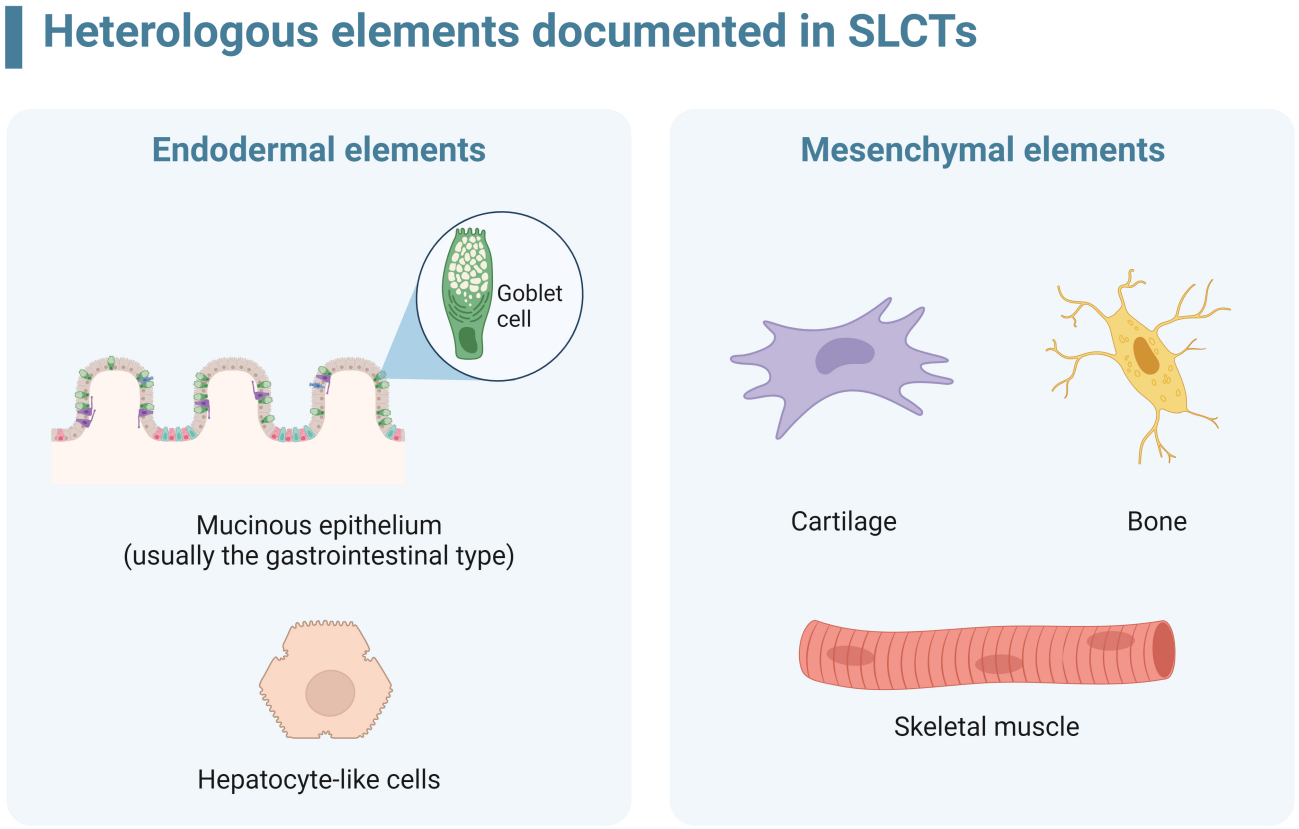

Fig. 3. Endodermal and mesenchymal heterologous elements that have been documented in SLCTs [4,9,26]. Created with BioRender.com (2023 BioRender, Science Suite Inc, Toronto, Ontario, Canada).

noid tumours, skeletal muscle or cartilage, and/or areas of rhabdomyosarcoma. Heterologous hepatocytes, retinal tissue and neuroblastoma have also been reported in some rare cases. Since hepatocytes and Leydig cells are morphologically similar, immunohistochemistry may be essential in the identification of heterologous hepatocytes. Positive immunostaining for α Fetoprotein (AFP), HepPar1, and arginase-1 affirms the hepatocytic nature of the cells [33]. Moreover, heterologous hepatocytes cause an elevated AFP in approximately 20% of patients [24].

Heterologous elements [26] are observable in approximately 20% of SLCTs, being found in all subtypes except for well-differentiated SLCTs [31]. As shown in Fig. 3 (Ref. [4,9,26]), these elements are divided into 2 categories depending on their origin: Endodermal elements and mesenchymal elements. Endodermal elements are more typically found within moderately differentiated SLCTs, whilst mesenchymal elements are more commonly found in poorly differentiated SLCTs [26]. Whereas endodermal elements do not usually affect the prognosis of the patient, mesenchymal elements have been shown to worsen the prognosis [34].

Genetic Findings

Keywords: DICER1, FOXL2, CYP19A1, Aromatase

Cytogenetic studies of ovarian SLCTs revealed karyotypic abnormalities affecting the sex chromosomes, including a case with X-chromosome mosaicism [35] and another case of a 46,XX karyotype with insertion of Y-chromosomal material into chromosome 1 [36]. Another cytogenic study of a metastasizing SLCT identified trisomy 8 as the only karyotypic abnormality [37]. Additionally, a cytogenic study of a virilising tumour revealed rearrangements in chromosomes 5 and 18, and trisomy 6 and 12 [38]. Another fluorescence *in situ* hybridisation (FISH) and comparative genomic hybridization (CGH) study of a SLCT revealed gain on chromosomes 19 and 22, and partial loss of chromosome 8 [39]. Although a number of chromosomal abnormalities have been discovered in SCSTs in general, their influence on pathogenesis remains unclear [40].

Karnezis *et al.* (2019) [41] identified 3 molecular subtypes of SLCTs in their study: *DICER1* mutant; *DICER1/FOXL2* wild type; and a novel *FOXL2* mutant subtype. Each molecular subtype has different clinicopathologic features, and those of the mutant subtypes are summarised and compared in Table 3 (Ref. [41]). Both *DICER1* and *FOXL2* mutations are thought to alter the expression of the Cytochrome P450 Family 1 Subfamily A Member 1

Table 3. A comparison of the clinicopathologic features of SLCTs with *DICER1* mutations and *FOXL2* mutations.

	DICER1 mutation	*FOXL2* mutation
Prevalence as found by Karnezis *et al.* (2019) [41]	44% of samples	19% of samples
Age at diagnosis	Adolescent women	Post-menopausal women
Effect on *CYP19A1* expression	Decreased expression	Increased expression
Clinical manifestations	Androgenic symptoms	Oestrogenic symptoms

DICER1, Dicer 1, Ribonuclease III; *FOXL2*, Forkhead Box L2; *CYP19A1*, Cytochrome P450 Family 1 Subfamily A Member 1.

Table 4. Possible differential diagnoses on inspection of SLCT microscopic features [34].

Differential diagnosis	Confounding microscopic features
Kurkenberg tumour	The presence of a tubular, sertoliform pattern. Sometimes there is a lack of prominent signet ring cells. Confusion of stromal lutein cells with Leydig cells. Stromal lutein cells stain for sex cord markers, and may lead to virilisation.
Mucinous cystic tumour	SLCTs with prominent mucinous elements.
Sarcoma	Sarcomatoid appearance of a poorly differentiated SLCT.
Mixed mesodermal tumour	SLCTs with a pattern of spindle cell sarcoma mixed with epithelial components.
Embryonal carcinoma	It can be confused with a very poorly differentiated SLCT.
Yolk sac tumour	Minor cysts in the SLCTs resemble a reticular–microcystic pattern. Oedematous appearance of an SLCT during pregnancy resembles a reticular pattern.

(*CYP19A1*) gene. However, it is still not clear whether the prognosis varies for each subtype, and hence whether clinical results correlate with mutation status. A trend has been observed where patients whose tumours had *DICER1* mutations were normally younger and more likely to present with androgenic manifestations [41]. Contrastingly, patients with *FOXL2* mutations were all postmenopausal and more likely to present with oestrogenic manifestations like abnormal bleeding. Hence, this is an example of how the underlying genetics of a tumour can affect the clinical presentation.

DICER1 Mutations

Well-differentiated SLCTs are *DICER1*-independent, but poorly and moderately differentiated SLCTs typically have an underlying *DICER1* mutation [18]. Karnezis *et al.* (2019) [41] found that 44% of their overall samples had somatic mutations in the RNase IIIb domain of *DICER1*, all of which showed moderate or poor differentiation. This percentage includes all cases presenting with heterologous elements or retiform patterns [41].

Both sporadic and germline *DICER1* mutations have been discovered in SLCTs. Germline mutations were first documented in families with pleuropulmonary blastoma, multinodular goitre (nodular hyperplasia of the thyroid), cystic nephroma and SLCTs of the ovary. Other conditions which have been linked to germline *DICER1* mutations include lung cysts, embryonal rhabdomyosarcoma of the uterine cervix, nodular carcinoma of the thyroid gland, pituitary blastoma, nasal chondromesenchymal hamartoma, renal sarcoma, ciliary body medulloepithelioma, Wilms tumour and pineoblastoma [42]. Co-existence of any of the mentioned conditions is highly indicative of *DICER1* syndrome, a rare genetic condition caused by *DICER1* germline mutations which predisposes one to hereditary cancer [9,43]. Apart from an increased risk of developing other cancers, SLCT patients with an underlying *DICER1* mutation have an increased risk of developing contralateral, metachronous ovarian tumours [44]. A metachronous tumour is a second primary cancer, diagnosed more than 6 months after the first diagnosis of primary cancer [29]. An example is a reported case of a woman with germline *DICER1* mutation who developed an ovarian undifferentiated sarcoma and a SLCT four years later [42]. Therefore, individuals with germline *DICER1* mutations should receive continued monitoring following the treatment of a SLCT.

Mutation frequencies in published studies vary considerably, ranging from 15% to 97% of tumours, with germline mutations making up to 69% of mutations. For instance, Hanley and Mosunjac (2019) [9] report that about 60% of patients presenting with moderately or poorly differentiated tumours have somatic *DICER1* mutations, whilst Karnezis *et al.* (2019) [41] found a considerably lower somatic *DICER1* mutation frequency of 41%. This variation in mutation frequencies is due to different variables among different studies. To date, *DICER1* mutations have never been observed in a grade 1 SLCT, so changing the proportion of well-differentiated tumours included in the study can affect the overall mutation frequencies. The mutation detection method used can also lead to different results, depending on whether a study used Sanger or next-generation sequencing, or both. In addition, the age of the selected patients is also significant as younger age at diagnosis is linked with *DICER1* mutations [41].

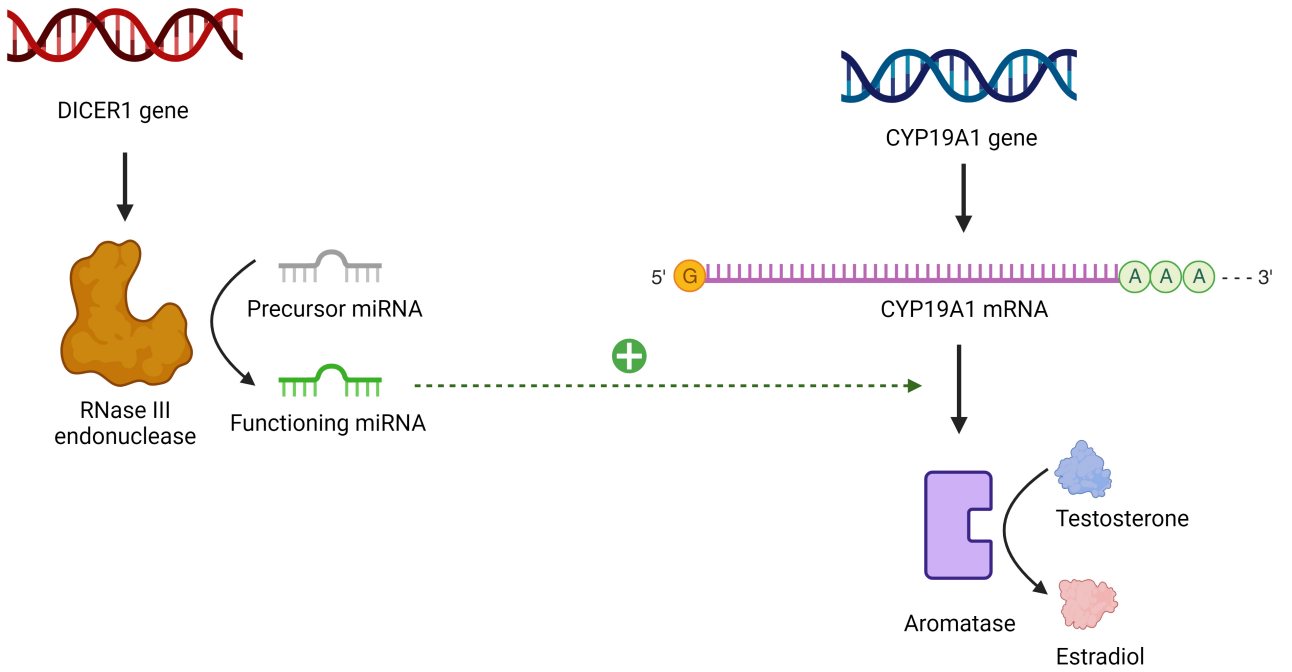

Fig. 4. The relationship between *DICER1* and *CYP19A1* gene expression. Created with BioRender.com (2023 BioRender, Science Suite Inc, Toronto, Ontario, Canada).

The tumourigenic mechanism of *DICER1* mutations is not clear [3]. *DICER1* is a gene that can be considered as either a tumour suppressor gene due to loss-of-function mutations or an oncogene due to gain-of-function mutations. Moreover, it may function as a haploinsufficient tumour suppressor gene. This means that the loss of a single allele results in tumour progression but the loss of both alleles has an inhibitory effect on tumour development, suggesting that one intact allele is required for the survival of the cell [45].

The majority of tumours in patients with *DICER1* syndrome have one allele containing a germline nonsense or frame-shift mutation, resulting in a total loss of function [42,46]. Schultz *et al.* (2016) [42] also found that the other allele contains a somatic missense mutation in the *DICER1* RNase IIIb domain. The combined presence of a germline mutation in one allele and a somatic mutation in the other allele results in improper cleaving of 5p miRNAs from precursor hairpin structures [47]. Nevertheless, there have also been some rare cases of biallelic somatic mutations in paediatric SLCT patients, in the absence of a germline mutation [42]. This supports the two-hit tumour suppression model in which *DICER1* acts as a tumour suppressor gene, rather than the haploinsufficient model. However, more recently, Yuan *et al.* (2020) [3] found that three SLCT patients had both germline and somatic mutations, whilst two other patients had either germline or somatic mutations but not both. This result suggests that the two-hit tumour suppression model and the haploinsufficiency model might both be at play [3].

The reason why androgenic manifestations are more common in SLCTs with *DICER1* mutations rather than wild-type *DICER1* is that those with *DICER1* mutations have lower mRNA levels for *CYP19A1*. The relationship between *DICER1* and *CYP19A1* gene expression is illustrated in Fig. 4. The *CYP19A1* gene encodes a member of the cytochrome P450 family; aromatase, which converts testosterone to estradiol. Therefore, low *CYP19A1* mRNA levels in tumours with a *DICER1* mutation leads to a decreased production of aromatase, and less testosterone is converted to estradiol, which explains the more frequent androgenic manifestations [48]. Two mechanisms leading to decreased *CYP19A1* mRNA expression have been suggested. The first hypothesis is that the *DICER1* mutations may directly inhibit the maturation of miRNAs that typically promote *CYP19A1* mRNA expression since the *DICER1* gene encodes an RNase III endonuclease which cleaves precursor microRNAs into functioning microRNAs [42]. The other hypothesis is that global miRNA expression is dysregulated by the protein product of the mutated *DICER1* gene, and this causes the ovarian cell to resemble a Sertoli cell. If this hypothesis is correct, the reduction in *CYP19A1* mRNA would be an indirect consequence of this differentiation to a more male phenotype rather than a direct consequence of the mutation [41].

FOXL2 Mutations

More recently, Karnezis *et al.* (2019) [41] found a somatic missense point mutation c.402C>G (p.C134W) in the *FOXL2* gene in 19% of their samples. The *FOXL2* gene encodes a forkhead transcription factor which is predominantly found in the ovary, the pituitary gland and the eyelids [49,50]. Similar to *DICER1* mutants, all the *FOXL2* mutant

samples were moderately or poorly differentiated. However, *FOXL2* mutations are mutually exclusive from the *DICER1* mutations, indicating that there is no pathophysiological link between the 2 variants [41]. This novel discovery is significant because the mutation concerned was considered to be pathognomonic for adult-type granulosa cell tumours (AGCT) [50].

FOXL2 mutations were found only in postmenopausal women who had abnormal bleeding as the most common presentation. Therefore, patients with *FOXL2* mutations present with oestrogenic symptoms, more similar to granulosa cell tumours than typical SLCTs. The oestrogenic manifestations of patients with *FOXL2* mutant tumours are most likely due to the mutant *FOXL2* transcription factor having the *CYP19A1* gene as a direct target and stimulating the overproduction of aromatase [51]. This leads to increased oestradiol levels, and thus oestrogenic symptoms. Overall, the occurrence of heterologous elements or a retiform pattern predicts *DICER1* mutation, and a low tumour grade predicts the absence of either a *DICER1* or *FOXL2* mutation [41]. Other characteristics such as age and presenting symptoms are not useful predictors in the clinical setting, even though they are correlated with mutation status [41]. Yuan *et al*. (2020) [3] conducted whole exome sequencing (WES) to explore the SLCT genetic landscape. This was the first study to investigate the rate of *DICER1* mutations in SLCTs of the Chinese population and also the first to investigate other genetic variations associated with the pathogenesis of SLCTs. Apart from observing the documented mutations in *DICER1* and *FOXL2*, mutations were also identified in *PALB2* and *PMS2* [3].

Clinical Presentation

Virilisation occurs in up to 60% of patients, primarily in those with moderately or poorly differentiated SLCT forms [52]. The incidence of virilization increases as tumour differentiation decreases. Patients with virilisation first show symptoms of defeminisation such as oligomenorrhea or amenorrhea, breast atrophy and loss of subcutaneous fat [19], followed by symptoms of masculinisation such as acne, hirsutism, clitoromegaly, a deepening voice, male-pattern baldness, increased libido and increased musculature [46,53].

Patients without virilisation are likely to present with non-specific signs like abdominal mass and/or distention and symptoms like pelvic pain [15]. Zhang *et al*. (2014) [13] found that 50% of SLCTs had no hormonal production and only presented with an adnexal mass or pain. This type of presentation is likely in retiform tumours or those with heterologous elements, since hormonal manifestations are uncommon features in these tumours [27].

Presentation due to excess oestrogen production is rare. Such patients would present due to postmenopausal bleeding, menorrhagia or metrorrhagia. Around 36% of postmenopausal women with SLCTs presented with symptoms of hyperoestrogenism [19]. Overall, the most common sign of an ovarian SLCT was irregular bleeding followed by an abdominal mass or amenorrhea [53].

Current Common Methods and Difficulties in Clinical Diagnosis

SLCTs might be detected on ultrasound if they are large enough. Assessment with ultrasonography might also show a thicker than normal endometrium, which is a sign of hyperoestrogenism. Computed tomography (CT), magnetic resonance imaging (MRI), and positron imaging tomography (PET) scans are other imaging modalities that can detect smaller SLCTs or metastatic spread [15].

Serum tumour markers can also be helpful in the detection of an SLCT. Serum testosterone levels which are higher than 5 nmol/L are associated with virilisation [54]. In patients with absent virilising symptoms, such as most patients with retiform SLCTs or those with heterologous elements, testosterone levels are likely to be normal. However, there might be raised oestrogens, raised serum AFP or raised serum cancer antigen 125 (CA-125) [27]. Nevertheless, Zhang *et al*. (2014) [13] report that only 80% of those SLCT patients with endocrine manifestations (either androgenic or oestrogenic) were found to have elevated testosterone or oestrogen. Hence, the absence of tumour markers cannot be used to exclude the diagnosis of a SLCT.

The microscopic features of SLCTs are of limited value when it comes to establishing a diagnosis, as a number of other tumours may mimic the microscopic features associated with SLCTs. This leads to a number of differentials, as shown in Table 4 (Ref. [34]). One differential is the Kurkenberg tumour, which refers to metastatic disease to the ovaries from a primary site, usually the gastrointestinal tract. Over 80% of these tumours are bilateral due to their metastatic origin, but monolateral disease is possible and more easily confused with a SLCT [55]. For improved diagnostic accuracy, Young (2018) [34] emphasizes the importance of taking multiple samples from different areas of the tumour. The patient's age is also relevant when interpreting the tumour sections [34]. If diagnosis is still difficult, a combination panel of immunohistochemistry (IHC) and haematoxylin and eosin (H&E) staining is the best way forward [34,53]. The most helpful IHC markers for recognizing a SLCT are negative staining for epithelial membrane antigen and positive staining for vimentin, inhibin and calretinin. Neural cell adhesion molecule 1 (CD56), *FOXL2*, *DICER-1*, Wilms' tumour gene 1 (*WT1*) and cluster of differentiation 10 (CD10) can also be helpful. However, the expression of markers of sex cord differentiation may be absent or minimal in poorly differentiated SLCTs, making their diagnosis more difficult [31].

Table 5. Recommended management for SLCTs of different FIGO stages and relapsed cases.

Stage	Surgery	Adjuvant chemotherapy
Young patients with FIGO I	USO in patients with well-differentiated stage Ia/Ib tumours (with or without assessment of the contralateral ovary), or if fertility needs to be conserved [13,58]. Patients with intermediately/poorly differentiated stage I tumours or tumours at stage Ic (rupture of capsule) could undergo USO only if followed by complete staging surgery [59].	Controversial for stage I patients, but recommended for those patients with poor prognostic factors such as intermediate/poor differentiation, heterologous elements, a retiform pattern, high mitotic activity and tumour rupture (stage Ic) [26,59].
Postmenopausal women with FIGO I	Abdominal hysterectomy and BSO with complete surgical staging is advised by ESMO if child-bearing is complete, due to unknown malignant potential [19].	Advised for patients with high-risk factors for tumour recurrence, such as FIGO stage IC, intermediate or poor differentiation, or the presence of heterologous elements or a retiform pattern [59–61].
FIGO II to IV	Abdominal hysterectomy and BSO with complete surgical staging [60].	Advised for all patients since FIGO stages II-IV have a high risk of recurrence [3] Limited data suggests radiotherapy as an alternative to chemotherapy, especially if the disease is limited to the pelvis [19].
	Cytoreductive surgery has been suggested [59].	HIPEC or HITOC [62,63].
Recurrence	Mostly radical surgery [10,62]. Other treatments are being investigated.	Platinum-based chemotherapy after surgery. Radiotherapy is useful in localized cases in which surgery is not an option [62].

BSO, bilateral salpingo-oophorectomy; ESMO, European Society for Medical Oncology; FIGO, International Federation of Gynaecology and Obstetrics; HIPEC, Hyperthermic Intraperitoneal Chemotherapy; HITOC, Hyperthermic Intrathoracic Chemotherapy; USO, unilateral salpingo-oophorectomy.

In most patients who present with an elevated serum AFP, the AFP-producing cells in the tumour are detected by IHC. In different SLCT case studies, AFP-positive IHC staining has been shown in Leydig cells only, in Sertoli cells only, in both Sertoli and Leydig cells, and in heterologous hepatoid cells [56]. Since Leydig cells and heterologous hepatocytes have similar morphology and have both been reported to show AFP positivity, additional IHC tests would be required to distinguish between them. Mooney *et al.* (1999) [57] reported that unlike Leydig cells, heterologous hepatocytes show positivity with keratin cocktails and CAM5.2, but are negative for vimentin and inhibin. Nevertheless, increased serum AFP and positive AFP staining are more frequently observed in ovarian yolk sac tumours, thereby complicating the diagnostic process with an additional differential [51].

Management

The gold standard of treatment is surgical resection, and the diagnosis is often made by pathology after surgery. From the stage of the disease, the tumour differentiation, the mitotic index, presence or absence of heterologous elements, and whether there has been capsular breach, pathologists can predict the likelihood of recurrence so that the appropriate measures can be taken in the management of the patient post-surgery [53]. Table 5 (Ref. [3,10,13,19,26,58–63]) describes the treatment options for patients with different needs and prognostic factors.

Surgery

Before surgery, serum markers are checked and the tumour is imaged, typically via transvaginal sonography, but other imaging modalities may be used if the tumour is too small or for staging in cases with metastatic spread [13,15]. The type of surgery depends on the age of the patient, stage of the tumour and degree of tumour differentiation [6].

Since most patients are diagnosed at reproductive age, fertility-sparing surgery must often be considered in patients with stage I or well-differentiated stage-II disease. Unilateral salpingo-oophorectomy (USO) is a conservative surgery which is performed [9]. However, conservative surgery is not offered to patients with higher stage disease due to a high risk of recurrence. In cases which are at FIGO stage 2 or higher and in cases in which fertility need not be conserved, total hysterectomy and bilateral salpingo-oophorectomy are recommended. It involves the removal of the uterus and cervix plus both ovaries and fallopian tubes [19]. The drawback of radical surgery is that it may lead to oestrogen depletion in young patients since hormone replacement therapy is contraindicated following a SLCT [62]. Gouy *et al.* (2019) [21] found that conservative surgery has a similar recurrence rate as radical surgery in stage Ia patients. However, the relapse rate for conservative surgery increases by about 30% in stage Ic disease. Therefore, it is imperative that the surgeon is extremely cautious to avoid a rupture when operating on young patients with an ovarian mass [21].

Gui *et al*. (2012) [59] also suggested cytoreductive surgery (CRS) for advanced stage disease. CRS consists of peritonectomy procedures and visceral resections with the goal of eliminating macroscopic disease. CRS is not effective in eliminating nodules smaller than 2.5 mm, so it is often combined with Hyperthermic Intraperitoneal Chemotherapy (HIPEC) or Hyperthermic Intrathoracic Chemotherapy (HITOC) to eliminate microscopic disease [64]. This method of treatment was originally used for epithelial ovarian tumours, but Larsen *et al*. (2020) [63] suggested that these principles can be used on an individualized basis in patients with metastatic SLCTs. Both USO and CRS can be performed by laparoscopy or laparotomy directly. Although the two procedures have similar surgical outcomes, laparoscopy allows for less blood loss, reduced operating time and a shorter period of hospitalization [13].

Adjuvant Chemotherapy

Adjuvant therapy is not recommended for patients with stage I and well-differentiated SLCTs, but postoperative chemotherapy is indicated for patients with poor prognostic factors [23]. The European Society for Medical Oncology (ESMO) guidelines on the management of non-epithelial ovarian tumours recommend adjuvant chemotherapy for SLCT patients with heterologous elements, poorly differentiated tumours or stage IB and IC disease [52]. Chemotherapy has also sometimes been recommended for patients with intermediately differentiated tumours [10].

Platinum-based chemotherapy is typically used for SCSTs [53]. Combination chemotherapy regimens for SLCTs have been applied through experience in treating other SCSTs. However, since SCSTs are so rare, there is no consensus on the ideal regimen. Currently, the bleomycin, etoposide and cisplatin (BEP) regimen is the most commonly used [65] and it is given for 3–4 cycles [63]. Alternative platinum-based regimens include Cisplatin, Adriamycin, and Cyclophosphamide (PAC); Cisplatin, Vinblastine, and Bleomycin (PVB); and paclitaxel-cisplatin [52].

Although adjuvant chemotherapy is routinely administered for patients with advanced stage disease, its usefulness is still not proven given the very low incidence of advanced stage SLCTs [10]. Two out of three patients with advanced disease who received chemotherapy had a recurrence [13]. Data obtained by Gui *et al*. (2012) [59] also suggests that adjuvant chemotherapy has limited benefits when administered after initial surgery, but is likely to be useful at relapse.

Post-Surgical Period and Follow Ups

Following surgical excision of the tumour, serum inhibin, androgen, testosterone, and oestrogen levels should return within normal limits [26]. Zhang *et al*. (2014) [13] found that in patients with androgenic manifestations, testosterone levels decreased to normal in the ten days following surgery. Feminine characteristics return rapidly, but the resolution of masculinisation features is slow [66]. In an analysis of 40 SLCT cases conducted by Gui *et al*. (2012) [59], patients who had already achieved menarche and who did not receive chemotherapy resumed normal menstruation within 1–3 months postoperatively. Among those who received chemotherapy, some had irregular bleeding throughout, while others returned to a regular menstrual cycle 6 months after stopping chemotherapy [59].

Patients should be followed up every three months during the first year, every four months during the second year, every six months during the third year, and annually from then onwards. At each follow-up visit, serum testosterone should be measured, and an abdominal and pelvic ultrasound performed. If required, a CT or MRI of the abdomen and pelvis may be requested [19].

Lifelong follow-up is necessary because although most recurrences occur within 36 months, there have been cases of recurrence as late as 35 years [13]. Additionally, those patients with germline *DICER1* mutations are at risk of developing contralateral metachronous ovarian tumours as well as other tumours associated with *DICER1* syndrome [10]. For this reason, genetic screening is also recommended. The most sensitive way of detecting germline or somatic *DICER1* mutations is somatic testing of tumour tissue [42]. If a germline mutation is detected, family members are to be tested and managed accordingly.

Management of Relapse Patients

Radical surgery combined with chemotherapy is recommended for SLCT relapses [62]. Conservative endocrine-sparing surgery may also be considered in those patients with a localized relapse and a normal contralateral ovary [67]. Moreover, secondary cytoreduction surgery and chemotherapy can be used to manage tumour recurrence, but poor results have been reported [10].

Alternative treatment options for relapse patients have been explored in the past, with some of these yielding good results and meriting further research. For example, since SLCTs express follicle-stimulating hormone (FSH) receptors, Lashkari *et al*. (2013) [68] gave long-acting gonadotrophin releasing hormone (GnRH) analogue to two relapse patients. This was based on the hypothesis that high GnRH promotes malignant transformation [69], as well as data which showed that FSH supports the growth of granulosa cell tumours [70]. Leuprolin was given to the patients for 2 years following surgery and adjuvant chemotherapy, and neither of them suffered tumour relapse [68].

Given that angiogenesis is involved in tumour development and progression, anti-angiogenic therapy with bevacizumab is thought to be useful. In 2014, the Gynaecologic Oncology Group (GOG) published a phase II trial which assessed the antitumour activity of bevacizumab in patients with recurrent SCSTs, and demonstrated that bevacizumab is effective and has an accepted toxicity [71].

Nonetheless, not one of the 36 tumours included in the original trial had SLCT histology. Therefore, the utility of bevacizumab in the management of SLCTs requires further investigation [10]. More recently, the GOG conducted a clinical trial which suggested that adding bevacizumab to paclitaxel for patients with relapsed SCST has no benefit. Nevertheless, it was reported that weekly paclitaxel alone stopped tumour progression for 6 months in 71% of patients, suggesting paclitaxel as another regimen option for relapsed SLCTs [72]. Currently, an active trial is testing the effectiveness and toxicity of paclitaxel with carboplatin relative to BEP for treating advanced or recurrent SLCTs (Gynaecologic Oncology Group NCT01042522).

Prognosis

Prognosis is determined mainly by stage and degree of differentiation, but also by tumour size and the presence of mesenchymal heterologous elements and/or a retiform pattern.

A Multicenter Trial on Ovarian Cancer (MITO) study carried out in Italy showed that a stage I tumour had a 92.3% 5-year survival rate, whilst 2/3 of patients with an advanced stage disease died [18]. Additionally, Rathi *et al.* (2015) [15] obtained similar survival rates, with the survival rate for stages III and IV specifically being 0%. Hence, these studies confirm the prognostic value of staging. The MITO study also confirmed the prognostic relevance of the tumour grade since the 5-year overall survival for grade 1 tumours was 100%, and the survival for patients with grades 2–3 was 77.8% [18]. In grade 2 and 3 tumours, the prognosis may be further worsened by the presence of a retiform pattern, heterologous elements of mesenchymal origin or a neuroblastoma [34]. Although endodermal elements do not usually worsen the prognosis [13], Yamamoto *et al.* (2019) [33] described the first case of a moderately differentiated SLCT with heterologous hepatocytes and widespread overgrowth of malignant transformed hepatocytes which affected prognosis.

Only a low percentage of SLCTs become clinically malignant since all well-differentiated tumours and most intermediately/poorly differentiated tumours are discovered at stage I [13]. Tumour stage and tumour size are significant as they present a greater risk of metastasis [65]. The tumour stage is based on extraovarian spread, and rupture or spillage of the tumour. Hanley and Mosunjac (2019) [9] report that extraovarian spread is seen in 2–3% of patients at the time of diagnosis.

Although previous studies have shown the malignant potential of SLCTs to be approximately 10–30% [65], the risk of recurrence must also be considered when dealing with the prognosis of the patient. SLCT relapses are typically systemic and multi-focal, making them difficult to treat [62]. Overall, about 20% of all SLCT patients have a relapse. Relapse tends to occur early, with 95% of recurrences occurring within 5 years of diagnosis [67]. As such, in many centres, SLCT patients are monitored for 2–5 years after surgery before they are considered to be at low risk for recurrence [42]. Nonetheless, there have been cases of recurrence after 35 years [13].

Table 6. Relapse rate and death rate at different stages of disease [3,21].

Stage	Rate of relapse	Death on relapse
IA	7.0%	70.0%
IC	30.0%	54.0%
II to IV	73.7%	78.6%

As already discussed, post-operative chemotherapy is given to patients with poorly or moderately differentiated tumours because they are associated with a higher risk of recurrence. In contrast, the recurrence rate for well-differentiated tumours after excision is virtually non-existent [15]. The presence of heterologous elements is also a poor prognostic factor which necessitates chemotherapy administration [65]. Finally, chemotherapy is given to patients with tumours which are FIGO stage IC or higher because, as shown in Table 6 (Ref. [3,21]), relapse is also more common in patients with advanced stage disease [3].

The prognosis of SLCTs is relatively good when compared to ovarian epithelial carcinomas [59], but worse than granulosa cell tumours [10]. Prognosis is poorer in SLCT patients without endocrine manifestations as the ratio of large tumours which are over 10 cm in diameter, tumour rupture (stage Ic), and tumours of poor differentiation is higher than those in androgenic or oestrogenic groups. This might be indicative of more aggressive pathophysiology in groups without endocrine function [59].

In summary, prognosis depends on both the malignant potential of the tumour and the risk of relapse. Additionally, both the stage and grade of tumour affect prognosis, and these are also related since grade 2 or 3 tumours are more likely to be diagnosed at more advanced stages. Furthermore, additional poor prognostic factors such as larger tumour size and the presence of heterologous elements or a retiform pattern are mostly associated with intermediate or poorly differentiated tumours rather than well-differentiated ones. Overall, well-differentiated SLCTs have the best prognosis as they are benign and do not recur, whilst the retiform variant is the subtype with the worst prognosis [15,18].

Challenges in Research and Future Direction

Future efforts should focus on finding the best management plan, particularly for advanced stage disease and relapse cases. More information about the molecular and genetic pathogenesis of the tumour can help improve tumour diagnosis and classification, prognosis and management. However, there are several challenges which are hindering the progress of research on rare gynaecological tu-

mours in general. One of the major challenges is the standardization of the clinical and histopathological guidelines to manage a rare gynaecological tumour (RGT). Such standardization is important, not only for diagnosing a specific RGT like a SLCT, but also for designing clinical trials for new therapies [73,74]. Currently, the significant diversity arising from how samples are handled across different centres (e.g., variations in processing and storage before analysis) is likely to lead to an overall lack of consistency and reliability in the results obtained from studies conducted at multiple centres. This, in turn, may hinder the ability to draw strong conclusions. Another challenge is the lack of availability of good quality tumour tissue for research. Hence, establishing specialized biobanks for RGT and defining Standard Operating Procedures (SOAPs), will be crucial in gathering a suitable collection of biological specimens accompanied by relevant clinical data for the development of personalized medicine approaches [75].

Actions taken to address these issues are scattered across different countries. International collaborations, such as the European Network for Gynaecological Rare Cancer Research (GYNOCARE), are needed in order to improve the diagnosis and treatment of rare gynaecological tumours by establishing a network between key stakeholders. The coordination of current and upcoming research, the establishment of virtual biobanks, and the harmonisation of the mandated legal standards in different European states (for facilitation of international trials) are important to bridge the gap between translational research and the pharmacological and biotechnology industries [76].

Conclusion

In conclusion, Sertoli-Leydig cell tumours (SLCTs) constitute a distinct subgroup within the rare spectrum of mixed sex cord-stromal tumours (SCSTs) in the ovary. Despite their infrequent occurrence, they hold significant clinical importance, particularly among young adults. Although challenges persist in the clinical diagnosis of SLCTs, the utility of immunohistochemistry has emerged as a valuable tool in enhancing diagnostic accuracy. Furthermore, the stratification of patients for treatment based on age, disease stage, and prognostic factors is integral to the comprehensive management of SLCTs. While surgical resection remains the cornerstone of treatment, the consideration of adjuvant chemotherapy, tailored to the individual risk of recurrence, has shown promising outcomes. The ongoing challenge of managing recurrence, coupled with the risk of metachronous tumour development, particularly in patients with *DICER1* syndrome, underscores the necessity for robust genetic testing and patient surveillance.

This comprehensive scoping review sheds light on the current understanding of SLCTs, emphasizing the need for continued research efforts to refine diagnostic modalities, treatment strategies, and long-term prognosis. As we advance into the future, a deeper understanding of the molecular intricacies and the establishment of specialized international biobanks will be pivotal in advancing the field and improving outcomes for individuals affected by SLCTs.

Availability of Data and Materials

Not applicable.

Author Contributions

JC-A contributed to the conception of the research work. CM designed the research study and performed the research. JC-A supervised the work. CM drafted the manuscript and JC-A revised it critically for important intellectual content. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Mills S. Histology for Pathologists. 5th edn. Lippincott Williams & Wilkins: Philadelphia. 2019.

[2] Sharma S, Valiathan M, Kumar S, Kapoor S. Ovarian Sertoli-Leydig Cell Tumour with Heterologus Elements Masquerading as Mucinous Tumour on Frozen Section: A Case Report. Journal of Clinical and Diagnostic Research: JCDR. 2015; 9: ED01–ED03.

[3] Yuan Z, Huo X, Jiang D, Yu M, Cao D, Wu H, *et al*. Clinical Characteristics and Mutation Analyses of Ovarian Sertoli-Leydig Cell Tumors. The Oncologist. 2020; 25: e1396–e1405.

[4] Horta M, Cunha TM, Marques RC, Félix A. Ovarian Sertoli-Leydig cell tumor with heterologous elements of gastrointestinal type associated with elevated serum alpha-fetoprotein level: an unusual case and literature review. Journal of Radiology Case Reports. 2014; 8: 30–41.

[5] Al Harbi R, McNeish IA, El-Bahrawy M. Ovarian sex cord-stromal tumors: an update on clinical features, molecular changes, and management. International Journal of Gynecological Cancer: Official Journal of the International Gynecological Cancer Society. 2021; 31: 161–168.

[6] Sachdeva P, Arora R, Dubey C, Sukhija A, Daga M, Singh DK. Sertoli-Leydig cell tumor: a rare ovarian neoplasm. Case report and review of literature. Gynecological Endocrinology: the Official Journal of the International Society of Gynecological Endocrinology. 2008; 24: 230–234.

[7] Meyer R. The pathology of some special ovarian tumors and

their relation to sex characteristics. American Journal of Obstetrics and Gynecology. 1931; 22: 697–713.

[8] Young RH. Sertoli-Leydig cell tumors of the ovary: review with emphasis on historical aspects and unusual variants. International Journal of Gynecological Pathology: Official Journal of the International Society of Gynecological Pathologists. 1993; 12: 141–147.

[9] Hanley KZ, Mosunjac MB. Practical Review of Ovarian Sex Cord-Stromal Tumors. Surgical Pathology Clinics. 2019; 12: 587–620.

[10] Nasioudis D, Mastroyannis SA, F Haggerty A, M Ko E, Latif NA. Ovarian Sertoli-Leydig and granulosa cell tumor: comparison of epidemiology and survival outcomes. Archives of Gynecology and Obstetrics. 2020; 302: 481–486.

[11] Edmund LN, Salama AM, Murali R. Cytologic features of sex cord-stromal tumors in women. Cancer Cytopathology. 2022; 130: 55–71.

[12] Young RH, Scully RE. Ovarian Sertoli-Leydig cell tumors. A clinicopathological analysis of 207 cases. The American Journal of Surgical Pathology. 1985; 9: 543–569.

[13] Zhang HY, Zhu JE, Huang W, Zhu J. Clinicopathologic features of ovarian Sertoli-Leydig cell tumors. International Journal of Clinical and Experimental Pathology. 2014; 7: 6956–6964.

[14] Guo L, Yang X, Zhu H, Qiu W, Shi X, Huang B, *et al*. Sertoli-Leydig cell tumor presenting hyperestrogenism in a postmenopausal woman: a case report and review of the literature. Taiwanese Journal of Obstetrics & Gynecology. 2012; 51: 620–624.

[15] Rathi M, Budania SK, Khalid M, Mittal A. Bilateral retiform variant of sertoli leydig cell tumour of ovary: An uncommon tumor with review of literature. Journal of Mid-life Health. 2015; 6: 35–38.

[16] Schultz KAP, Harris AK, Finch M, Dehner LP, Brown JB, Gershenson DM, *et al*. DICER1-related Sertoli-Leydig cell tumor and gynandroblastoma: Clinical and genetic findings from the International Ovarian and Testicular Stromal Tumor Registry. Gynecologic Oncology. 2017; 147: 521–527.

[17] Nwogu LC, Showalter JA, Roy S, Deavers MT, Zhao B. Retiform Sertoli-Leydig Cell Tumor in a 38-Year-Old Woman: A Case Report, Retrospective Review, and Review of Current Literature. Case Reports in Pathology. 2017; 2017: 3421832.

[18] Guo Y, Wang J, Li Y, Wang Y. Ovarian Sertoli-Leydig cell tumors: an analysis of 13 cases. Archives of Gynecology and Obstetrics. 2020; 302: 203–208.

[19] Priyadarshini M, Dixit J, Ramaswamy V, Mannan C, Deepak HL, Rastogi P. Ovarian Sertoli-Leydig Cell Tumor with Estrogenic Manifestations in a Postmenopausal Lady: a Case Report. Indian Journal of Surgical Oncology. 2019; 10: 499–501.

[20] Bhat RA, Lim YK, Chia YN, Yam KL. Sertoli-Leydig cell tumor of the ovary: analysis of a single institution database. The Journal of Obstetrics and Gynaecology Research. 2013; 39: 305–310.

[21] Gouy S, Arfi A, Maulard A, Pautier P, Bentivegna E, Leary A, *et al*. Results from a Monocentric Long-Term Analysis of 23 Patients with Ovarian Sertoli-Leydig Cell Tumors. The Oncologist. 2019; 24: 702–709.

[22] Durmuş Y, Kılıç Ç, Çakır C, Yüksel D, Boran N, Karalök A, *et al*. Sertoli-Leydig cell tumor of the ovary: Analysis of a single institution database and review of the literature. The Journal of Obstetrics and Gynaecology Research. 2019; 45: 1311–1318.

[23] Khalloufi C, Joudar I, Kanas A, Benhessou M, Ennachit M, El Kerroumi M. Ovarian Sertoli-Leydig tumor: A tricky tumor case report. International Journal of Surgery Case Reports. 2023; 105: 108043.

[24] Monappa V, Reddy SM, Kudva R, Pai MV. Cystic Sertoli-Leydig Cell Tumour in a Postmenopausal Woman with Absent Virilising Symptoms: A Diagnostic Challenge. Journal of Clinical and Diagnostic Research: JCDR. 2017; 11: ED26–ED28.

[25] Goldblum JR, Lamps LW, McKenney J, Myers JL. Rosai and Ackerman's Surgical Pathology. 11th edn. Elsevier: Philadelphia. 2017.

[26] Sahoo TK, Kar T, Kar A, Panda S. Poorly Differentiated Sertoli-Leydig Cell Tumour of Ovary with Heterologous Elements. Journal of Clinical and Diagnostic Research: JCDR. 2017; 11: XD01–XD02.

[27] Scott IV. Diagnostic Pathology of Ovarian Tumors. Journal of Obstetrics and Gynaecology (Lahore). 2012; 32.

[28] Abu-Zaid A, Azzam A, Alghuneim LA, Metawee MT, Amin T, Al-Hussain TO. Poorly differentiated ovarian sertoli-leydig cell tumor in a 16-year-old single woman: a case report and literature review. Case Reports in Obstetrics and Gynecology. 2013; 2013: 858501.

[29] Lv M, Zhang X, Shen Y, Wang F, Yang J, Wang B, *et al*. Clinical analysis and prognosis of synchronous and metachronous multiple primary malignant tumors. Medicine. 2017; 96: e6799.

[30] Gautam P, Rao M, Gothwal M, Garg PK, Bhattacharya S. Sertoli-Leydig Cell Tumor of Ovary: A Rare Case Report with Heterologous Elements and Focal Marked Anaplasia. International Journal of Applied & Basic Medical Research. 2019; 9: 62–64.

[31] Lim D, Oliva E. Ovarian sex cord-stromal tumours: an update in recent molecular advances. Pathology. 2018; 50: 178–189.

[32] Young RH. Sex cord-stromal tumors of the ovary and testis: their similarities and differences with consideration of selected problems. Modern Pathology: an Official Journal of the United States and Canadian Academy of Pathology, Inc. 2005; 18 Suppl 2: S81–S98.

[33] Yamamoto S, Sakai Y. Ovarian Sertoli-Leydig Cell Tumor with Heterologous Hepatocytes and a Hepatocellular Carcinomatous Element. International Journal of Gynecological Pathology: Official Journal of the International Society of Gynecological Pathologists. 2019; 38: 247–252.

[34] Young RH. Ovarian sex cord-stromal tumours and their mimics. Pathology. 2018; 50: 5–15.

[35] Hitosugi M, Matsuoka Y, Irimajiri S, Fukuda J. Sertoli-Leydig cell tumour complicated by X chromosomal mosaicism. Clinical Endocrinology. 1997; 47: 619–622.

[36] De Giorgi U, Turci D, Crisanti E, Cantù MG, Cappellini A, Marangolo M. Ovarian Sertoli-Leydig cell tumor, endometrioid-like yolk sac tumor, and Y-chromosomal material. Oncology. 2003; 64: 370–373.

[37] Manegold E, Tietze L, Günther K, Fleischer A, Amo-Takyi BK, Schröder W, *et al*. Trisomy 8 as sole karyotypic aberration in an ovarian metastasizing Sertoli-Leydig cell tumor. Human Pathology. 2001; 32: 559–562.

[38] Truss L, Dobin SM, Rao A, Donner LR. Overexpression of the BCL2 gene in a Sertoli-Leydig cell tumor of the ovary: a pathologic and cytogenetic study. Cancer Genetics and Cytogenetics. 2004; 148: 118–122.

[39] Verdorfer I, Horst D, Höllrigl A, Rogatsch H, Mikuz G. Sertoli-Leydig cell tumours of the ovary and testis: a CGH and FISH study. Virchows Archiv: an International Journal of Pathology. 2007; 450: 267–271.

[40] Fuller PJ, Leung D, Chu S. Genetics and genomics of ovarian sex cord-stromal tumors. Clinical Genetics. 2017; 91: 285–291.

[41] Karnezis AN, Wang Y, Keul J, Tessier-Cloutier B, Magrill J, Kommoss S, *et al*. DICER1 and FOXL2 Mutation Status Correlates with Clinicopathologic Features in Ovarian Sertoli-Leydig Cell Tumors. The American Journal of Surgical Pathology. 2019; 43: 628–638.

[42] Schultz KAP, Harris A, Messinger Y, Sencer S, Baldinger S, Dehner LP, *et al*. Ovarian tumors related to intronic mutations in DICER1: a report from the international ovarian and testicular stromal tumor registry. Familial Cancer. 2016; 15: 105–110.

[43] Caroleo AM, De Ioris MA, Boccuto L, Alessi I, Del Baldo G,

Cacchione A, *et al*. DICER1 Syndrome and Cancer Predisposition: From a Rare Pediatric Tumor to Lifetime Risk. Frontiers in Oncology. 2021; 10: 614541.

[44] Goulvent T, Ray-Coquard I, Borel S, Haddad V, Devouassoux-Shisheboran M, Vacher-Lavenu MC, *et al*. DICER1 and FOXL2 mutations in ovarian sex cord-stromal tumours: a GINECO Group study. Histopathology. 2016; 68: 279–285.

[45] González IA, Stewart DR, Schultz KAP, Field AP, Hill DA, Dehner LP. DICER1 tumor predisposition syndrome: an evolving story initiated with the pleuropulmonary blastoma. Modern Pathology: an Official Journal of the United States and Canadian Academy of Pathology, Inc. 2022; 35: 4–22.

[46] De Paolis E, Paragliola RM, Concolino P. Spectrum of *DICER1* Germline Pathogenic Variants in Ovarian Sertoli-Leydig Cell Tumor. Journal of Clinical Medicine. 2021; 10: 1845.

[47] Pugh TJ, Yu W, Yang J, Field AL, Ambrogio L, Carter SL, *et al*. Exome sequencing of pleuropulmonary blastoma reveals frequent biallelic loss of TP53 and two hits in DICER1 resulting in retention of 5p-derived miRNA hairpin loop sequences. Oncogene. 2014; 33: 5295–5302.

[48] Kato N, Kusumi T, Kamataki A, Tsunoda R, Fukase M, Kurose A. DICER1 hotspot mutations in ovarian Sertoli-Leydig cell tumors: a potential association with androgenic effects. Human Pathology. 2017; 59: 41–47.

[49] Georges A, Auguste A, Bessière L, Vanet A, Todeschini AL, Veitia RA. FOXL2: a central transcription factor of the ovary. Journal of Molecular Endocrinology. 2013; 52: R17–R33.

[50] Carles A, Trigo-Gonzalez G, Cao Q, Cheng SWG, Moksa M, Bilenky M, *et al*. The Pathognomonic FOXL2 C134W Mutation Alters DNA-Binding Specificity. Cancer Research. 2020; 80: 3480–3491.

[51] Fleming NI, Knower KC, Lazarus KA, Fuller PJ, Simpson ER, Clyne CD. Aromatase is a direct target of FOXL2: C134W in granulosa cell tumors via a single highly conserved binding site in the ovarian specific promoter. PLoS ONE. 2010; 5: e14389.

[52] Morgan RJ, Jr, Armstrong DK, Alvarez RD, Bakkum-Gamez JN, Behbakht K, Chen LM, *et al*. Ovarian Cancer, Version 1.2016, NCCN Clinical Practice Guidelines in Oncology. Journal of the National Comprehensive Cancer Network: JNCCN. 2016; 14: 1134–1163.

[53] Weng CS, Chen MY, Wang TY, Tsai HW, Hung YC, Yu KJ, *et al*. Sertoli-Leydig cell tumors of the ovary: a Taiwanese Gynecologic Oncology Group study. Taiwanese Journal of Obstetrics & Gynecology. 2013; 52: 66–70.

[54] Hirschberg AL. Approach to Investigation of Hyperandrogenism in a Postmenopausal Woman. The Journal of Clinical Endocrinology and Metabolism. 2023; 108: 1243–1253.

[55] Aziz M, Killeen RB, Kasi A. Krukenberg Tumor. StatPearls Publishing: Treasure Island (FL). 2023.

[56] Jang K-T. Alpha-Fetoprotein Producing Sertoli-Leydig Cell Tumor of the Ovary - A Case Report. Journal of Pathology and Translational Medicine. 2002; 36: 128–131.

[57] Mooney EE, Nogales FF, Tavassoli FA. Hepatocytic differentiation in retiform Sertoli-Leydig cell tumors: distinguishing a heterologous element from Leydig cells. Human Pathology. 1999; 30: 611–617.

[58] Negri S, Grassi T, Fruscio R. Use of staging for sex cord stromal tumours. Current Opinion in Oncology. 2022; 34: 504–510.

[59] Gui T, Cao D, Shen K, Yang J, Zhang Y, Yu Q, *et al*. A clinicopathological analysis of 40 cases of ovarian Sertoli-Leydig cell tumors. Gynecologic Oncology. 2012; 127: 384–389.

[60] Ray-Coquard I, Morice P, Lorusso D, Prat J, Oaknin A, Pautier P, *et al*. Non-epithelial ovarian cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology. 2018; 29: iv1–iv18.

[61] Schneider DT, Orbach D, Cecchetto G, Stachowicz-Stencel T, Brummel B, Brecht IB, *et al*. Ovarian Sertoli Leydig cell tumours in children and adolescents: An analysis of the European Cooperative Study Group on Pediatric Rare Tumors (EXPeRT). European Journal of Cancer. 2015; 51: 543–550.

[62] Nef J, Huber DE. Ovarian Sertoli-Leydig cell tumours: A systematic review of relapsed cases. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2021; 263: 261–274.

[63] Larsen NK, Reilly MJ, Kwon S, Loggie BW, Balters MW. Cytoreduction with hyperthermic intra peritoneal and intra thoracic chemotherapy for metastatic Sertoli-Leydig cell tumor of the ovary. Gynecologic Oncology Reports. 2020; 34: 100638.

[64] Mehta SS, Bhatt A, Glehen O. Cytoreductive Surgery and Peritonectomy Procedures. Indian Journal of Surgical Oncology. 2016; 7: 139–151.

[65] Liggins CA, Ma LT, Schlumbrecht MP. Sertoli-Leydig cell tumor of the ovary: A diagnostic dilemma. Gynecologic Oncology Reports. 2015; 15: 16–19.

[66] Stegner HE, Lisboa BP. Steroid metabolism in an androblastoma (Sertoli-Leydig cell tumor): a histopathological and biochemical study. International Journal of Gynecological Pathology: Official Journal of the International Society of Gynecological Pathologists. 1984; 2: 410–425.

[67] Seidler SJ, Huber A, Nef J, Huber DE. Sertoli-Leydig Cell Ovarian Tumors: Is Fertility or Endocrine-Sparing Surgery an Option upon Relapse? Case Reports in Oncology. 2020; 13: 935–940.

[68] Lashkari HP, Nash R, Albanese A, Okoye B, Millar R, Pritchard-Jones K. Treatment of high risk Sertoli-Leydig cell tumors of the ovary using a gonadotropin releasing hormone (GnRH) analog. Pediatric Blood & Cancer. 2013; 60: E16–E18.

[69] Stadel BV. Letter: The etiology and prevention of ovarian cancer. American Journal of Obstetrics and Gynecology. 1975; 123: 772–774.

[70] Davy M, Torjesen PA, Aakavaag A. Demonstration of an FSH receptor in a functioning granulosa cell tumour. The effect of gonadotrophin treatment on its viability following transplantation to nude mice. Acta Endocrinologica. 1977; 85: 615–623.

[71] Brown J, Brady WE, Schink J, Van Le L, Leitao M, Yamada SD, *et al*. Efficacy and safety of bevacizumab in recurrent sex cord-stromal ovarian tumors: results of a phase 2 trial of the Gynecologic Oncology Group. Cancer. 2014; 120: 344–351.

[72] Ray-Coquard I, Harter P, Lorusso D, Dalban C, Vergote I, Fujiwara K, *et al*. Effect of Weekly Paclitaxel with or Without Bevacizumab on Progression-Free Rate Among Patients with Relapsed Ovarian Sex Cord-Stromal Tumors: The ALIENOR/ENGOT-ov7 Randomized Clinical Trial. JAMA Oncology. 2020; 6: 1923–1930.

[73] Lainé A, Hanvic B, Ray-Coquard I. Importance of guidelines and networking for the management of rare gynecological cancers. Current Opinion in Oncology. 2021; 33: 442–446.

[74] Ray-Coquard I, Trama A, Seckl MJ, Fotopoulou C, Pautier P, Pignata S, *et al*. Rare ovarian tumours: Epidemiology, treatment challenges in and outside a network setting. European Journal of Surgical Oncology: the Journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2019; 45: 67–74.

[75] Di Fiore R, Suleiman S, Ellul B, O'Toole SA, Savona-Ventura C, Felix A, *et al*. GYNOCARE Update: Modern Strategies to Improve Diagnosis and Treatment of Rare Gynecologic Tumors—Current Challenges and Future Directions. Cancers. 2021; 13: 493.

[76] GYNOCARE n.d. Available at: https://gynocare.net/ (Accessed: 12 February 2023).