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Preconceptional counseling of women with previous third and fourth degree perineal tears

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INTRODUCTION

More than 85% of women in the United Kingdom (UK) sustain some form of perineal trauma during childbirth¹. In the majority of instances, only the perineal skin, vaginal epithelium and superficial muscles are involved, and such tears are only rarely associated with serious sequelae. Tears involving the anal sphincter, however, can have long-term impact on a woman's quality of life. Sultan² originally proposed the classification of obstetric anal sphincter injuries (OASIS) shown in Figure 1; this classification was later adopted by the Royal College of Obstetricians and Gynaecologists³ and subsequently internationally accepted. A schematic representation of the anal sphincter is depicted in Figure 2. The

prevalence of third and fourth degree tears appears to be dependent upon the type of episiotomy practised and thus varies considerably. In centers where mediolateral episiotomy is practised, the rate of OASIS is 1.7% (2.9% in primiparas), compared to 12% (19% in primiparas) in units that perform midline episiotomy⁴.

This chapter describes the salient points that should be covered at the preconceptional consultation of a woman who has sustained a third or fourth degree OASIS. What this chapter does not cover specifically are the risk factors of anal sphincter injuries, evidence on how to prevent such injuries at first vaginal delivery, methods of repair of severe perineal tears and management of anal incontinence (whether surgical or medical).

First degree:	laceration of the vaginal epithelium or perineal skin only.
Second degree:	involvement of the perineal muscles but not the anal sphincter.
Third degree:	disruption of the anal sphincter muscles which should be further subdivided into: 3a: <50% thickness of external sphincter torn. 3b: >50% thickness of external sphincter torn. 3c: Internal sphincter also torn.
Fourth degree:	a third degree tear with disruption of the anal epithelium as well.

Figure 1 Classification of perineal trauma

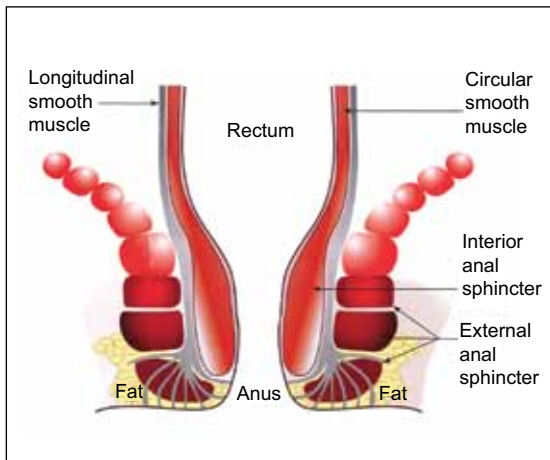


Figure 2 Schematic representation of the anal canal (modified from Sultan, Thakar and Fenner, 2009⁴)

DIRECTED HISTORY FOLLOWING OBSTETRIC ANAL SPHINCTER INJURY

OASIS is associated with both short- and long-term sequelae to young, otherwise healthy women. Although the issue of anal incontinence (defined as the loss of normal control of bowel action leading to the involuntary passage of flatus and/or feces) predominates in the literature, it is by no means the only effect of anal sphincter damage. Pain, infection, dyspareunia and sexual dysfunction also may be present and play important roles in the woman's emotional and psychological well-being. This section describes the effects of OASIS on bowel function and the questions that should be asked in order to elicit the extent of the problem. An accurate description of the events leading to the injury and details of the repair are important and are most easily obtained by perusing the patient's prior delivery related notes. Unfortunately, these are not always sufficiently complete as to be truly informative. Anal incontinence, the main long-term effect of OASIS, can range from a minor leakage of feculent fluid, through occasional passage of stool during passage of flatus, to complete loss of bowel control.

Approximately 3–5%⁵ of women experience fecal incontinence postpartum, but often are reluctant to discuss their symptoms. Bugg and colleagues⁶ distributed questionnaires on urinary and anal incontinence to 275 primiparous women 10 months after delivery. Although up to 10% of women had developed new symptoms of fecal incontinence, the authors noted that only a small proportion had raised the issue with their doctor or midwife.

Because of the intimate nature of this topic, information is better obtained by a questionnaire rather than direct questioning. Specific inquiries that should be incorporated in the questionnaire include⁷:

- How often do you open your bowels per week?
- Is your stool hard or soft?
- Is your passage of stool painful?
- Do you strain excessively to open your bowels?
- Do you feel you emptied yourself completely?
- Is there any mucus or blood in the stool?
- Are you able to control your stool?
- Can you tell the difference between stool and flatus (wind)?
- Do you lose flatus when you do not mean to?
- Do you have any leakage of loose stool?
- How often do you have loose stool per week or day?
- Do you wear pads?
- Do you feel stool coming and you are unable to stop it? (urge incontinence)
- Do you feel it after it is too late? (true incontinence).

Women who have sustained OASIS should be assessed by a senior obstetrician and detailed debriefing of the circumstances surrounding

the delivery offered and any concerns explored. A genital examination should be performed, looking for scarring, residual granulation tissue and tenderness. At this point, specialist investigations organized to assess anal function, as alluded to in the next section, may be considered.

INVESTIGATION OF ANAL INCONTINENCE

In order to complement the information gained at history taking and physical examination, a number of investigations may be ordered. Some of these may not be available in a generalist setting and referral to a specialist center may be necessary. Prior to this, the pathophysiology of anal incontinence is briefly considered to better understand the choice of investigations.

Observational studies of women suffering from anal incontinence postpartum identified two types of insults: mechanical (i.e. relating to muscle/anal sphincters) and neuropathic (i.e. relating to the nerve supply of the muscles). Injury to the pudendal nerve (which innervates the anal sphincters) as it courses over the pelvic floor, may result in suboptimal continence. The effect of this type of insult is also thought to be cumulative and worsens with subsequent pregnancies. The evidence for the above observation comes from studies of women who had cesarean sections performed either electively or in early labor (control group) compared to women who had a cesarean section as an emergency in late labor^{8,9}.

The following tests help to assess the structure and the physiological function of the anal sphincter.

Anorectal manometry

Anorectal manometry¹⁰ includes a series of measurements designed to establish:

- Deficits in anal sphincter function
- The presence or absence of rectoanal reflexes
- Rectal sensory function
- Defecatory function.

The apparatus consists of four components:

- An intraluminal pressure-sensing catheter
- Pressure transducers
- A balloon for inflation within the rectum
- The recording system.

No universally accepted standards exist for the equipment and/or the technique, unfortunately. Thus, it is difficult to compare data between centers, and it remains to each unit to develop its own normogram, preferably sex and age stratified. Anal sphincter function is assessed by identifying the functional anal canal length and by recording the maximum resting canal pressure and voluntary anal squeeze pressure. The length of the functional anal canal is shorter in incontinent patients than in control subjects; low resting anal tone is associated with passive anal incontinence, and low anal squeeze pressure correlates with symptoms of urge or stress fecal incontinence. Having said this, two potentially confounding factors operate in manometry: first, large diameter probes can distort the anal canal and falsely record high pressures; and, second, pressures in different areas vary at different levels of the anal cavity. Under these circumstances, calculated pressures should be averaged out.

The rectoanal contractile reflex (which can be assessed by manometry) is recruited at instances when the intrarectal pressure increases above the anal pressure. To prevent fecal leakage, the anal pressure should always exceed the intrarectal pressure; therefore, when increased intrarectal pressure is present, e.g. coughing, a multisynaptic response results in contraction of the external anal sphincter

maintaining the desired high anal pressure. In case of damage to the sphincter (whether structural or neurological), the reflex is lost and continence is compromised.

The purpose of assessing normal rectal sensation is to establish the ability of an individual to determine rectal distension and, therefore, the need to defecate. Rectal sensation may be quantified by using balloon distension, and the patient may be categorized into the rectal hypo- or hypersensitivity group. In general, it is believed that patients with rectal hypo-sensitivity may experience passive (overflow) incontinence, while hypersensitivity may present as urge incontinence. Anal manometry also allows the examiner to reproduce a situation simulating diarrhea by infusing large volumes of normal saline into the rectum. Accurate measurement of the volume of saline infused and leaked provides good evidence regarding rectal capacity and compliance. Patients with fecal incontinence are able to retain as little as 500ml of saline compared to a normal subject whose rectum can hold over 1500ml without any significant leakage.

Imaging of the anal sphincter

With the advent of detailed ultrasound and other imaging modalities, the radiologic depiction of the perineum and the anal sphincter complex has improved considerably over the years, and imaging is presently considered essential in the assessment and management of fecal incontinence, especially that related to obstetric trauma¹¹.

Imaging of the anal sphincters is achieved using an endoanal linear ultrasound probe and the different layers depicted are:

- Subepithelium, which is moderately reflective
- Internal anal sphincter, which shows low reflectivity

- Longitudinal layer, which is low to moderately reflective and consists of pubocervical fascia, smooth muscle from the longitudinal layer of rectum and striated muscle of the perineum
- External sphincter, which is of low to moderate reflectivity and can be divided into:
 - a subcutaneous part, which starts at the termination of the internal sphincter
 - a superficial part, which forms a complete ring around the anal canal
 - a deep part.

Figure 3 shows the normal four layer pattern of the anal canal on axial endosonography. Vaginal delivery affects sphincter morphology (even in the absence of OASIS); similar changes are not evident in pregnancy following elective cesarean section. Frudinger and colleagues¹² compared anal endosonography findings in nulliparous and age matched multiparous patients, demonstrating thinning of the anterior external sphincter with

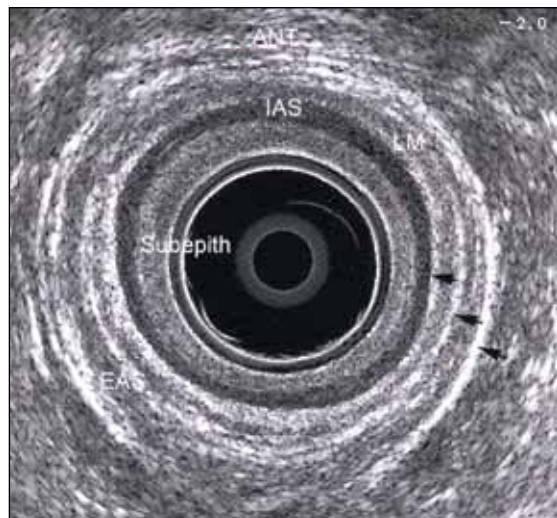


Figure 3 The normal pattern of the anal canal, as depicted by endoanal ultrasound. IAS, internal anal sphincter; EAS, external anal sphincter; ANT, anterior aspect; LM, longitudinal muscle

concurrent thickening of the longitudinal layer and the superficial external sphincter. These findings suggest diffuse trauma to the anus during delivery.

The use of anal endosonography has revolutionized the diagnosis of partial or small sphincter tears missed during clinical examination. A recent meta-analysis of tears demonstrated on ultrasound following 717 vaginal deliveries showed that the incidence of obstetric anal sphincter tears was as high as 26.9% in nulliparous and 8.5% for subsequent deliveries¹³. Injury of the external sphincter leads to formation of avascular scar tissue of uniform low reflectivity, which is easily detected on ultrasound as it crosses the planes.

The routine use of anal endosonography immediately after delivery has been investigated by Faltin and colleagues¹⁴, who randomized 752 primiparous women with second degree lacerations into a control group which had conventional vaginal examination and a study group which was assigned to additional postpartum endoanal ultrasonography. Interestingly, 5.6% of women in the study group (who were thought to have second degree tears) were found to have sustained severe OASIS. Severe fecal incontinence (as assessed by Wexner Incontinence Score) was reported 3 months postpartum by 3.3% of women in the study group compared to 8.7% in the control and these effects persisted 1 year after delivery. However, ultrasonography needed to be performed in 29 women to prevent one case of 'missed' severe fecal incontinence, and five women would have had unnecessary intervention, as the defect was not clinically demonstrated.

Even though endoanal ultrasonography is considered the gold standard for the morphological assessment of the anal canal, the lack of volume calculations and relative patient discomfort limit its use in clinical practice. New methods that have been introduced include transvaginal ultrasonography (a reliable method with accuracy equivalent to that of the

endoanal technique), transperineal ultrasound and endoanal magnetic resonance imaging (MRI). More recently, three-dimensional ultrasound has joined the field of pelvic floor imaging, and Valsky and colleagues¹⁵ prospectively studied 117 primiparous women without clinically recognized third and fourth degree anal tears at 24–72 hours postpartum using this modality. They found that the internal sphincter was visualized in 100% of patients, while the external sphincter was fully visualized in only 84.6% of patients. Since three-dimensional ultrasound allows the digital storage of volume, employing this method shortens the examination time (mean examination time 3.5 min) and permits the visualization of the sphincter in the 'resting' state.

Pudendal nerve terminal motor latency

Prior to the advent of endoanal ultrasound, pudendal nerve electrophysiological studies were widely used in case of suspected incontinence, as it was believed that the majority of idiopathic or neurogenic fecal incontinence cases were due to pudendal neuropathy. Pudendal nerve terminal motor latency (PNTML)¹⁰ measures the conduction time from stimulation of the pudendal nerve at the level of the ischial spine to the external anal sphincter contraction, with increased latencies being present in incontinent women following OASIS. However, the validity of the test has recently come under scrutiny, as both the sensitivity and specificity are poor and recent consensus advocates that the test should not be part of mainline investigations.

MANAGEMENT OF SUBSEQUENT PREGNANCIES

Figure 4 shows an algorithm for the management of women who had sustained OASIS in the previous pregnancy.

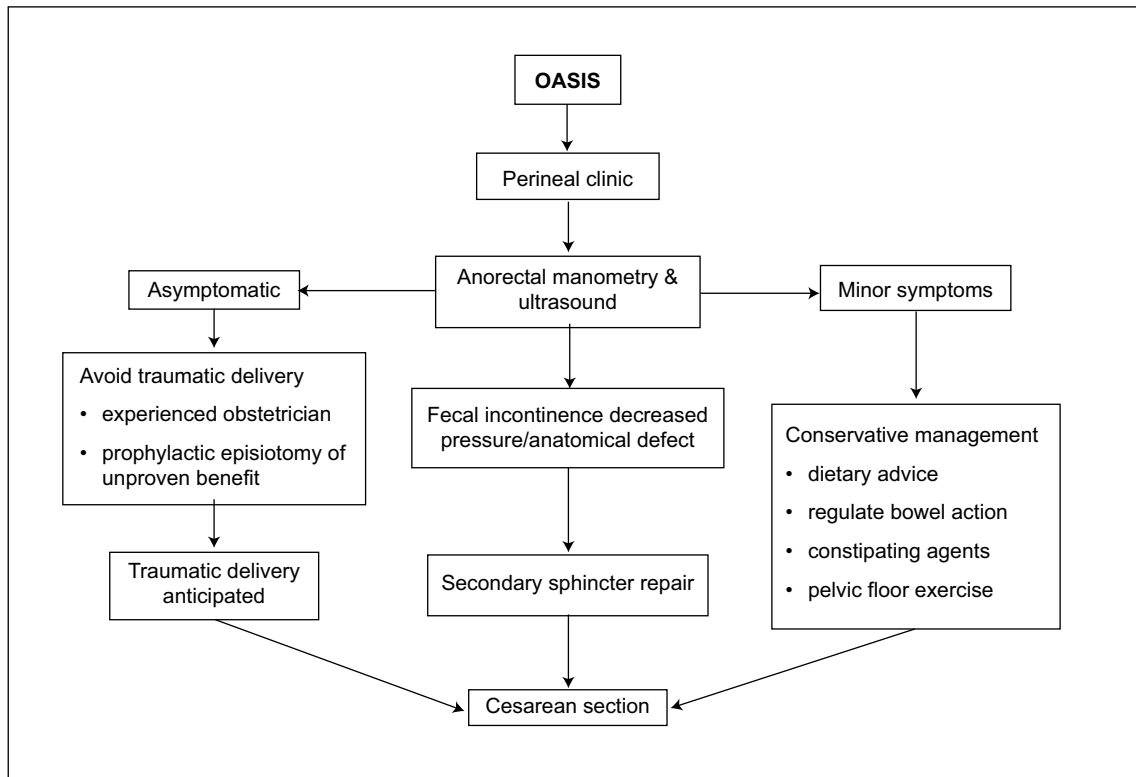


Figure 4 Flow diagram for the management of obstetric anal sphincter injury in subsequent pregnancies

Candidates for cesarean section

Women with mild fecal symptoms should be managed conservatively for their anal incontinence and a prelabor cesarean section advised in an attempt to prevent any further compromise to the sphincter function. Women with anal incontinence should be referred to a colorectal surgeon to consider secondary repair of the sphincter prior to conception and they should be offered a prelabor cesarean section. Fecally incontinent women who wish to consider vaginal delivery can have the secondary repair delayed until they have completed their family.

However, cesarean section is by no means the panacea of obstetrics, and fecal incontinence will not be eliminated, even if all women are delivered abdominally. A mail survey of a cohort of volunteers was conducted in France to estimate obstetric risk factors on

fecal incontinence among middle aged women (2640/3000 questionnaires were returned). The prevalence of fecal incontinence was similar in nulliparous, primiparous and multiparous women. Among parous women, this was similar in women with spontaneous vaginal, instrumental or cesarean section deliveries (9.3%, 10% and 6.6%, respectively). Lal *et al.*¹⁶ compared the anal function of 184 women who delivered via cesarean section with 100 women who delivered vaginally 10 months postnatally and found that reported symptoms of anal incontinence were similar between the two groups (5% vs 8%, $p > 0.05$).

Candidates for vaginal delivery

As a general rule, OASIS women who are asymptomatic with satisfactory anal pressure measurements and ultrasound imaging should

be counseled that they have a 95% chance of not sustaining recurrent anal sphincter injury or developing new symptoms following a subsequent vaginal delivery.

There is limited evidence regarding the methods that should be employed during labor to minimize recurrence of OASIS during vaginal delivery. The majority of data come from studies on nulliparous women and the results should be extrapolated with caution to women who have previously sustained OASIS.

Antenatal perineal massage

In a randomized study, Shipman *et al.*¹⁷ prospectively studied the effect of perineal self massage with almond oil in 861 nulliparous women 6 weeks prior to delivery and found a 6.1% reduction in second or third degree perineal tears in the study compared to the control group, especially in the over 30 years of age cohort. Similar results were noted by Labrecque and colleagues¹⁸ (who reported a 9.2% decrease in perineal trauma in nulliparous women practising perineal self massage), although this effect did not persist in subsequent vaginal deliveries. By combining the results of the above mentioned studies, Thakar *et al.*¹⁹ calculated that one case of perineal trauma requiring suturing would be avoided for every 13 nulliparous women who performed perineal massage antenatally. The risk of sustaining OASIS was found to be non-significant.

Birth position

Considerable discussion exists regarding the influence of position during the second stage of labor on perineal trauma. Eason *et al.*²⁰ reported an increase in the risk of perineal trauma requiring suturing in the upright birth position (using supporting furniture) compared to recumbent positions (weighted risk

difference 2%). Retrospective evidence from a study by Gareberg and colleagues²¹ described the risk of sustaining a third degree tear as seven times higher in the standing group, and the lateral (side lying) position has been suggested as the most protective posture to the perineum. However, these observations come from small randomized controlled trials, and larger studies are needed before drawing any conclusions and altering clinical practice.

Second stage pushing advice

Fraser and colleagues²² investigated the effects of early versus delayed pushing by randomizing 1862 women at full dilatation (with epidural *in situ*) to either actively push early or delay pushing unless the urge was too strong or the baby visible. They hypothesized that waiting at least 2 hours before actively pushing would reduce the risk of difficult operative delivery, as measured by cesarean section and midpelvic or low pelvic instrumental delivery rates. Delayed pushing reduced the number of difficult operative deliveries; this was associated with a decrease in cord pH but no difference on third or fourth degree tears was observed.

Use of episiotomy

For years routine episiotomy was encouraged in the belief that it reduced perineal trauma, allowed better healing than tears, and prevented pelvic floor relaxation. In 2000, a Cochrane review²² showed that routine episiotomy caused more posterior perineal trauma and was associated with more complications compared to selective episiotomy. More recently, Eason *et al.*²⁰ calculated that avoiding routine episiotomy in five women would prevent one case of perineal trauma requiring suturing with the weighted risk difference in anal sphincter tears being -1% (95% CI -1-0%). An interesting retrospective cohort

study on the influence of episiotomy at first vaginal delivery on the risk of perineal laceration in a subsequent vaginal delivery was published by Alperin and colleagues²⁴ who identified 6052 women with consecutive vaginal deliveries of live-born term singletons with vertex presentation. Of these, 47.8% had had an episiotomy at first delivery, and the rate of second degree lacerations at the time of second delivery was 51.3% in women with history of episiotomy compared to 26.7% in those without ($p < 0.001$). In addition, third and fourth degree tears occurred in 4.8% of women with previous episiotomy in contrast to 1.7% of controls. These authors extrapolated that for every four women in whom episiotomies were not performed in the first pregnancy, one second degree laceration would be prevented, thus suggesting that the consequences of performing an episiotomy can be perpetuated to subsequent vaginal deliveries presumably because scar tissue has less elasticity.

Use of instruments for operative vaginal delivery

Compelling evidence on the protective effect of vacuum extraction compared to forceps delivery led the Royal College of Obstetricians and Gynaecologists in the UK to recommend that the former should be the instrument of choice for operative vaginal birth. Literature review²⁵ suggests that one anal sphincter tear is avoided for every 18 women delivered by vacuum extraction instead of forceps (weighted risk difference -6% , 95% CI -8% to -4%).

RISK OF RECURRENCE

The evidence available on this issue is conflicting and appears related to the type of episiotomy practised in the delivery centers. Payne *et al.*²⁶ (who practised in a center that performed

midline episiotomy) prospectively followed 1741 women who had two consecutive vaginal deliveries and reported the recurrence rate to be 10.7% (19 out of 178 cases with a prior sphincter tear, adjusted OR 3.4). The same authors described the occurrence rate of third or fourth degree tears with the first delivery to be 10.2%, an unusually high percentage which was probably attributable to the type of episiotomy conducted.

Peleg and colleagues²⁷ found 704 sphincter injuries in 4015 primiparous deliveries; in these women, the recurrence rate of severe perineal tear was 2.1% when no episiotomy was performed, 11% with a midline episiotomy and 21% in instrumental deliveries with midline episiotomy. In another retrospective American cohort study²⁸ of 6068 women, a 7.2% recurrence rate of severe perineal laceration was reported compared to 2.3% in women who had a primary anal sphincter laceration. Statistical analysis of the significant risk factors contributing to the above figures included midline episiotomy (OR 8.5), vertex malpresentation (OR 4.3), shoulder dystocia (OR 2.7) and infant birth weight > 3500 g.

It is difficult to extrapolate the above data (from units that perform midline episiotomy) to centers in the UK and Europe where mediolateral episiotomy is routinely practised. Harkin and colleagues²⁹ reported a series of 20,111 consecutive vaginal deliveries from Dublin in which mediolateral episiotomy was performed, noting that 2.9% of primiparous and 0.8% of multiparous women sustained primary OASIS and that such a severe injury recurred in 4.4% of women. These researchers concluded that although OASIS was increased five fold at next delivery, 95% of women delivering vaginally after previous third and fourth degree tear did not sustain further sphincter damage.

RISK OF ANAL INCONTINENCE

Few studies have tried to establish the risk of anal incontinence in a subsequent vaginal

delivery following OASIS. Bek and Laurberg³⁰ studied 56 women who sustained OASIS and then had subsequent vaginal delivery; 52% of them remained asymptomatic following the second delivery, and only four of 23 women who had transient symptoms after the first delivery reported persistent symptoms after the birth of their second baby. Four women of the original 56 had persistent symptoms of anal incontinence following OASIS and interestingly, three of the four women denied any worsening of their symptoms after the second vaginal birth. Conversely, Poen and colleagues³¹ noted that the rate of anal incontinence was 56% in women who had a subsequent vaginal delivery following previous OASIS compared to 34% in those who did not subsequently deliver.

The role of the internal sphincter damage and rectal extension in the development of anal incontinence was highlighted in a study by Sangalli and colleagues³². They found that the rate of anal incontinence was 25% following fourth degree tears compared to 11.5% in women who sustained a third degree tear ($p = 0.049$).

Irrespective of the decision that women make for their subsequent delivery, they should be fully aware of the overall risks each mode of delivery entails. For example, cesarean section carries an 11.3% risk of maternal morbidity compared to 4.2% following vaginal delivery³³.

PREDICTIVE FACTORS

Being able to predict the women who would develop anal incontinence following OASIS would prove extremely valuable in counseling and managing patients, but any such evidence from prospective studies is sparse.

Fynes *et al.*³⁴ followed 59 women who had two consecutive vaginal deliveries at three different time points; 34 weeks' gestation of their first pregnancy and 6–12 weeks postnatally after two consecutive vaginal deliveries. Women with transient fecal incontinence or

occult anal-sphincter injury after their first vaginal delivery are at high risk of fecal incontinence after a second vaginal delivery (75% vs. 5% of women with less extensive defects, $p < 0.0001$).

Starck and colleagues³⁵ carried out an informative longitudinal study, whereby 41 women who suffered a third or fourth degree tear were recruited and followed with anal manometry and endoanal ultrasonography at 1 week, 3 months and 1 year postpartum. The women were asked to complete a questionnaire pertaining to bowel function (Wexner Score) 1 and 4 years after delivery. The most predictive variable of the Wexner score at 4 years was the endoanal sonographic sphincter defect score at 1 week ($r = 0.48$, $p = 0.002$).

AGE-RELATED INCONTINENCE SYMPTOMS

The progression of anal incontinence symptoms over time has recently attracted clinical interest. Fornell and colleagues³⁶ invited 82 cases and controls (who had participated in a previous prospective study on third degree tears) back to their clinic 10 years after delivery. The patients were asked to complete identical questionnaires on anal incontinence and sexual symptoms to those completed 10 years previously and all underwent anal manometry and endoanal ultrasonography. Incontinence of flatus and liquid stool was more severe in cases than in controls, a finding which was consistent over the years. In women with OASIS, the maximal squeeze pressures were significantly lower at 10 years compared to at 6 months postinjury (as recorded during the initial prospective study). Internal sphincter injury demonstrated by endoanal ultrasonography was associated with significantly more severe incontinence symptoms. Perineal body thickness was similar irrespective of the degree of sphincter tear, but women with perineal bodies < 10 mm had more vaginal dryness and

worse flatular incontinence. Of interest was the fact that 10% of the women in the OASIS group had undergone secondary repair of the anal sphincter because of fecal incontinence.

Samarasekera *et al.*³⁷ investigated the long-term anorectal function and quality of life in three groups of women: group 1 consisted of women who had sustained a third degree tear 10 years prior ($n = 54$); group 2 included the next delivered patient with an uncomplicated vaginal delivery ($n = 71$); and group 3 those who delivered by elective cesarean section ($n = 54$). Women in group 1 had significantly higher rates of anal incontinence and impaired quality of life scores compared to women in groups 2 and 3 ($p < 0.0001$). Similarly, mean resting and squeeze pressures were lower and sphincter defects were more persistent in the study group compared to the two control groups ($p < 0.05$).

Thus, in women who had sustained OASIS, symptoms of anal incontinence as well as quality of life scores may worsen as age and menopause related changes set in.

MEDICOLEGAL PROBLEMS

It is often difficult for a claimant to discharge a burden of proof that a third or fourth degree tear occurred through a lack of skill or care³⁸. The usual accusation is that either OASIS was 'missed' or the repair was suboptimal. Up to a third of women having their first vaginal delivery may sustain occult sphincter injury³⁹, and 'missed' mechanical disruption of the anal sphincter is increasingly recognized as a major contributor to subsequent fecal incontinence.

A consensus statement arising from the conference 'Obstetric anal sphincter injury: Is it time to rethink practice as we enter the Millennium?' held in Birmingham, UK, in 2000 recommended that any woman having had an *instrumental delivery* or *sustaining a perineal tear* should undergo digital rectal examination by an individual trained in the recognition of

third degree tears: failure to recognize a significant sphincter injury by not conducting a rectal examination is the principle cause of successful litigation.

A recent meta-analysis indicated that 66% of women remain fully continent of feces and flatus after primary repair and 49% after secondary repair⁴⁰. It follows that the repair of an OASIS must be executed with appropriate skill and care, and the consensus statement further advises that the procedure should be performed by a 'trained professional' in a well equipped and lit operating theater following a set protocol.

In the preconceptional counseling of women who have had previous third or fourth degree perineal tears, risk management and potential litigation become important issues in the group with previous OASIS who elect to have vaginal births. Informed consent is paramount, and it is crucial that all these women are carefully examined by digital rectal examination following delivery and that any repair be conducted according to protocol by experienced trained personnel.

CONCLUSION

Women with previous fecal incontinence who have had successful repair should have prelabor cesarean section. Women with OASIS who are asymptomatic with satisfactory anal pressure measurements and ultrasound imaging should be counseled that they have a 95% chance of not sustaining a recurrence in a subsequent vaginal birth, although anal incontinence may still arise with increasing age. Risk management and potential litigation are important issues in the group with previous OASIS who elect to have vaginal deliveries. Informed consent is paramount and it is crucial that all these women are carefully examined by digital rectal examination following subsequent delivery and that any repair be conducted according to protocol by experienced trained personnel.

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