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Supplementary Material

Supplementary Material 1: Detailed description of cryptorchidism risk factors relating to maternal health.

There is some evidence of an association between **maternal gestational diabetes** and cryptorchidism– with a recent meta-analysis of 12 studies showing a 20% increased risk (pooled OR: 1.21, 95% CI 1.00-1.46).[100] However, the authors of the meta-analysis also observed a moderate degree of heterogeneity between studies (I-squared 57.3%) – a reflection of the fact that while some studies have found an association between gestational diabetes and cryptorchidism, [208 87 48] many have not.[80 209 210 82 89 81 46 211 92,212] Perhaps the most convincing observations were made by Trabert et al.,[211] who

used routinely-collected laboratory data on diabetes status from a large cohort (n=150,144 mother/son pairs) and observed no association between gestational diabetes and cryptorchidism development (adjusted OR: 0.93, 95% confidence interval 0.77-1.10).

There is evidence of an association between **preeclampsia/pregnancy-induced hypertension** and cryptorchidism.[48 82 89 68] For example, Jones et al. [48] observed a 24% increased risk of orchidopexy-confirmed cryptorchidism among sons born to mothers with preeclampsia compared to those without (adjusted OR: 1.24, 95% CI 1.06-1.45), while McGlynn et al. observed a 70% increased risk (adjusted RR: 1.7, 95% CI 1.1-2.6). Jones et al. suggest that rather than causing cryptorchidism, preeclampsia may be a marker for other causal factors, including poor placental function.[48]

Studies have reported either weak[82] or no [83 78] evidence of an association between cryptorchidism and **proteinuria**, an indicator of renal dysfunction that often accompanies preeclampsia – although there is limited evidence of increased likelihood of **other renal pathology** among mothers of cryptorchid sons, as well as **gallstones**.[88,89] There is no evidence of an association between cryptorchidism and **glucosuria** (glucose in urine),[78] **anemia** [81] or degree of **weight gain during pregnancy**.[82]

There is conflicting evidence regarding an association between **nausea and vomiting** among pregnant woman and risk of cryptorchidism: while some studies have observed increased risk among women who reported vomiting during pregnancy, [79] others have found either no association [68 213 83] or a protective association.[76 78 214] There is some evidence [76 79] that severe and/or prolonged vomiting during pregnancy (*hyperemesis gravidarum)* might be associated with cryptorchidism; however, there is also evidence to the contrary [82 214] – and given the rarity of both cryptorchidism and hyperemesis gravidarum (1-2% of pregnancies[82]), this association is difficult to test, and due to low prevalence hyperemesis gravidarum could only be a risk factor for a small proportion of cryptorchidism cases.

There is conflicting evidence regarding **bleeding during pregnancy** and risk of cryptorchidism: while some authors have observed an association,[68 215] others have not.[76 79 83] This exposure has been inconsistently measured across studies –those studies reporting an association between bleeding and cryptorchidism tended to use a more severe definition of bleeding than those that reported no association. For example, Zhang et al. [215] observed that mothers who experienced threatened spontaneous abortion (i.e. severe bleeding during early pregnancy) had nearly three times the odds of their baby having cryptorchidism compared to those without such bleeding (crude OR among full-term pregnancies: 2.7, 95% CI 1.4-5.1). If there were a causal association, the direction of causality is unclear. Thus, Zhang et al. [215] suggested that threatened abortion may reflect a natural biological mechanism by which the body aborts an abnormal embryo; however it may reflect commonality between the causes of testicular maldescent and the causes of severe maternal bleeding.[215]

One study has observed an association between the **common cold** (with or without fever) among mothers during early pregnancy and development of cryptorchidism in the son (crude OR: 1.8, 95% CI 1.0-3.0);[216] however the authors suggest that this observation may have been confounded by medications taken to treat a cold (as discussed elsewhere in this review), and it is important to note that this association is based on an observation from a single study.

Recently, Ingstrup et al. [217] investigated the impact of recent bereavement (as a measure of **intense emotional distress** among pregnant woman) on the risk of cryptorchidism – based on an animal

models that have shown that maternal stress can alter testicular descent.[218] However, the authors found no association with testicular maldescent in humans (adjusted OR: 1.02, 95% CI 0.92-1.14).[217] Pierik et al. [75] observed a strong association between poor **self-reported general health** among mothers and cryptorchidism among sons (adjusted OR: 3.8, 1.5-9.8), as well as a strong protective association between a **vegetable-rich diet** and cryptorchidism (OR: 0.4, 95% CI 0.2-0.9); however, these observations were made in a single study from a relatively small cohort, based on self-reported data on exposures collected (on average) 11 weeks after birth after the diagnosis of crytoorchidism and hence which may have been subject to reporting bias.[75]

Non-pregnancy-related health conditions

There is some evidence of an association between **pre-conception endometriosis** and development of cryptorchidism in the son. Mavrogenis et al. [110] observed that the sons of mothers who had a medically-recorded occurrence of endometriosis were more than twice as likely to develop cryptorchidism than the sons of mothers who did not have this condition (adjusted OR: 2.42, 95% CI 1.71-3.42). However, given the lag between pre-conception endometriosis and maldescent of the testes within the developing foetus, the authors suggest that this association is likely to reflect shared causal factors between endometriosis and development of cryptorchidism in the son rather than endometriosis causing cryptorchidism.[110]

There is little [187] [81] or no [76] [100] [79] [219] [82] [6] evidence of an association between **pre-pregnancy BMI** (or being **overweight/obese**) and risk of cryptorchidism in the son. There is also little [89] [81] or no [82] [78] evidence that **chronic hypertension** is associated with cryptorchidism.

Two studies have investigated the role of **blood type** on cryptorchidism risk, with conflicting results [84] [8] – and no clear causal mechanism identified.

Supplementary Material 2: Additional information regarding candidate genes associated with cryptorchidism development.

Variants in more than 15 genes have thus far been implicated in the development of cryptorchidism in humans via candidate studies (AhR,[201] AR,[220] ARNT2,[201] AXIN1, [221] CYP17A1,[201] ESR1,[222 223 224] HOXA10,[225] INSL3,[226] LRG3/GREAT,[226,227] NR1I2,[201] PDE4B,[228] RXFP2,[229] SF-1,[230] SPAG5,[231] and STRBP [232]), many of which have clear biological plausibility in terms of the aetiology of cryptorchidism. However, there appears to be considerable variability in the current state of evidence in terms of the genes that have been associated with cryptorchidism across different contexts, and the direction of the association in those cases where the same gene has been implicated across different contexts. With respect to the first point, several authors have observed that the relative importance of a given gene differs substantially between populations/regions: for example, Qin et al. [201] looked at polymorphisms across 15 different genes and compared allele frequencies between an Italian cohort and a Japanese cohort, and found that none of the polymorphisms that were positively associated with cryptorchidism were common to both the Italian and Japanese cohorts.[201] With respect to heterogeneity of effects across studies, Yoshida et al. [223] observed a significant positive association between cryptorchidism and polymorphisms at the Estrogen Receptor 1 (*ESR1*) gene in Japan – while in Italy, Galan et al. [224] observed a significant protective association for this gene. The cause of this divergence remains unclear.

Supplementary Material 3: Additional information regarding post-birth risk factors for testicular ascent/descent.

Breastfeeding

As discussed elsewhere in this review, analysis of breast milk has been used in the literature as a proxy for maternal – and, by extension, foetal and/or neonatal – chemical exposure during the perinatal period. Some authors have also investigated the role of breastfeeding (or lack thereof) *per se* as a factor in the development of cryptorchidism. In 1992, Mori et al. [78] observed that Japanese boys who were not breastfed had more than three-times the odds of requiring orchidopexy to correct their cryptorchidism (crude OR: 3.5, 95% CI 1.2-10.2), while Jones et al. [48] later observed that boys who were primarily fed formula rather than breast milk were more likely to have orchidopexy-confirmed cryptorchidism (adjusted OR: 1.18, 95% CI 1.00-1.39). More recently, Barthold et al. [234] investigated the role of feeding method on the risk of cryptorchidism, and observed that boys fed primarily with formula were more likely to 'acquire' cryptorchidism (ascent of testes after birth) compared to those primarily fed with breast milk (adjusted ORs: non-soy formula 1.8, 95% CI 1.1-2.9; soy-based formula 2.7, 95% CI 1.4-5.4), but were no more likely to be born with cryptorchidism.[234] (The latter was also observed by Davies et al., who observed similar rates and duration of breastfeeding among boys born with cryptorchidism compared to boys born without cryptorchidism.[46])

This compelling observation suggests that a non-congenital exposure – i.e. reduced breastfeeding and/or exposure to non-breast milk, particularly soy-based formula – may contribute to testicular ascent/maldescent following birth; however, the aetiological mechanism behind this association remains unclear. Barthold et al. [234] suggest that it is possible that hormones present in breast milk play a contributing role in securing the final position of the testes following birth, and that reducing

the availability of these hormones – while replacing them with non-maternal substitutes – may affect success in this respect. Further work is required to determine whether breastfeeding and cryptorchidism are linked directly (i.e. causally) or indirectly (i.e. via shared risk factors [234]).