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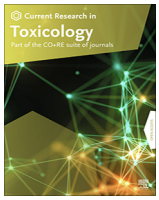

Toxic effects of smokeless tobacco on female reproductive health: A review

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ABSTRACT

The habitual consumption of tobacco in its various form is widespread and a serious public health issue globally. In particular, the use of smokeless tobacco has increased substantially due to its easy availability and misconception that it is relatively harmless compared to smoking. Tobacco use has been well established from numerous studies as a causative agent of devastating illnesses such as cancer, insulin resistance, hypertension, acute respiratory disease, osteoporosis, etc. Limited but growing evidence have also suggested its role in adversely affecting reproductive capabilities and outcomes in women of reproductive age and during pregnancy. This paper provides an updated review on available literature regarding the negative effects of smokeless tobacco use on female reproductive health, during pregnancy and its adverse consequences on the offspring. Existing data suggests the association between chronic smokeless tobacco use and impairment of ovarian morphology and function, oocyte quality, hormonal perturbations, fetal development and long-term health effects on the fetus. Improved understanding of these issues can contribute to better awareness of the dangers of smokeless tobacco products.

1. Introduction

The epidemic of tobacco use in various forms has been a critical global health issue with devastating consequences and its numerous negative health impacts have been well established. According to the World Health Organisation, tobacco-related diseases were responsible for about 8 million deaths in 2017 alone, and it has also projected that this number would increase to about 1 billion in the 21st Century if there are no positive changes in the current trends. Although smoking is still the primary method of tobacco use, the trends of smokeless tobacco (ST) consumption has also seen a sharp rise in many parts of the world partly due to the misconception that it is a safer alternative to smoking (Siddiqi et al., 2017). ST is a broad term that includes a variety of tobacco products that can be consumed as alternatives to smoking, and are consumed either orally and nasally and the types may vary from region to region. The most prevalent ones include 'chewing tobacco' which are dried tobacco leaves, 'snuff' which consists of finely ground dry or moist tobacco, 'mishri' which is baked or burnt ground tobacco and '*gutkha*' which is a mixture of tobacco with slaked lime, areca nut, catechu (acacia plant extract) and spices (Willis et al., 2014; Niaz et al., 2017). It has been estimated that globally, about 356 million people in 140 countries consume ST products in varied forms (Sinha et al., 2018). The main constituents of ST include nicotine and recognized carcinogens such as tobacco-specific N-nitrosamines (TSNA), benzo[a]pyrene, nitrate, cadmium, lead, arsenic, nickel, and chromium (Borgerding et al., 2012, Hecht, 2019; Guan et al., 2021). Studies have shown that higher levels of nicotine can enter systemic circulation from ST when compared to smoking, indicating a much more potent effect through this route (Saleheen et al., 2014, Li et al., 2018). Benowitz (1997) reported that from chewing tobacco, about 4.5 mg nicotine was systemically absorbed from an average dose of 7.9 g chewed for 30 min and an average of 3.6 mg nicotine was absorbed from 2.5 g moist snuff kept in the mouth for 30 min which are evidently much higher than absorption amounts from smoking cigarettes where an average of 1.0 mg nicotine was absorbed per cigarette. The harmful impacts of tobacco use have been intensely investigated and majority of research has focussed on smoking and ST-use related disorders such as lung cancer, oral squamous cell carcinoma, cardiovascular disease, immunosuppression, insulin resistance, hypertension, endocrine disruption etc. (Kuper et al., 2002; Kapoor and Jones, 2005; Willis et al., 2012; Mouhamed et al., 2016; Kim et al., 2019; Jiang et al., 2019). A detailed review by Beal et al. (2017) has pointed to the far-reaching genetic consequences of tobacco use in the form of heritable changes that could be passed on to their offspring. In addition to these conditions, tobacco has been known to exert its negative influence on reproductive physiology and outcome in both males and females. In females in particular, chronic tobacco use has been linked to impairment of fertility and disruptions at various stages of reproductive function such as ovotoxicity, disturbed hormone levels, abnormal delivery, complications during

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pregnancy, stillbirths and miscarriages, indicating harmful *in utero* effects on pregnant women (Kapoor and Jones, 2005, Kumar, 2013; Budani and Tiboni, 2017; Jandikova et al., 2017). The Global Adult Tobacco Survey (GATS) reported in 2007 that 6.7% of women in the 16 countries they surveyed consumed ST in one form or another (Giovino et al., 2012). In spite of prevalence of use and its established adverse effects, comprehensive and detailed reviews in this aspect, particularly in regards to ST use are still lacking. Taking this lacuna into consideration, in this review, an attempt has been made to assess the multifaceted effects of ST use on female fertility and reproductive outcomes. Available literature on ST use and its associated adversative effects on female reproductive health were searched on Pub Med and Google scholar and reviewed. A list of various types of ST products, their general constituents and where they are most prevalently used is given in Table 1. The paper is divided into various sections based on the effects of ST on the different aspects of female reproduction, and both human and animal studies are included. The toxic effects of ST on female reproductive physiology have been summarized in Table 2.

2. Chemical constituents of ST

More than 3000 compounds have been identified in ST thus far, and the exact constituents are influenced by the region from which the tobacco in sourced, types of pesticides used and the methods employed to process the leaves (IARC, 2007). Alkaloids are the major compounds present in tobacco (0.5–5.0%) out of which nicotine is the chief constituent (85–95% of total alkaloids) and a variety of other compounds are also found in the form of polyphenols (0.5–4.5%), terpenes (0.1–3.0%), phytosterols (0.1–2.5%), carboxylic acids (0.1–0.7%), alkanes (0.1–0.4%), aromatic hydrocarbons, aldehydes, ketones, amines, nitriles, N- and O-heterocyclic hydrocarbons, pesticides, alkali nitrates (0.01–5%) and numerous metallic compounds (IARC, 2004). Nicotine present in ST products can be absorbed mainly in two forms: protonated and unprotonated, and it has been shown that unprotonated/free nicotine, the amount of which is affected by the pH of the product, can effectively cross cell membranes (Pickworth et al., 2014). The nitrosation of nicotine as a result of metabolism gives rise to TSNAs, where N'-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone are the two most predominant forms and have been classified as group 1 carcinogens. N-nitrosamino acids [includes N-nitrososarcosine, 3-(methylnitrosamino) propionic acids and 4-(methylnitrosamino) butyric acids] and volatile N-nitrosamines [includes N-nitrosodimethylamine, N-nitrosopyrrolidine, N-nitrosopiperidine and N-nitrosomorpholine] are other classes of nitroso compounds present in ST products (IARC, 2007; Kaur et al., 2018; Hecht, 2019). Apart from these, additives such as menthol, benzyl benzoate, eugenol, benzo[a]pyrene (BaP), urethane, heavy metals, lactones and pesticides are also present in ST products with varying concentrations (IARC, 2007; Willis et al., 2012; Song et al., 2016; McAdam et al., 2018). One study by McAdam et al. (2017) evaluating the radionuclide content of 34 Swedish snus and 44 US ST products detected 13 radionuclides such as ^{40}K, ^{14}C, ^{210}Po and ^{226}Ra in the majority of sam-

Table 1
Some common types of smokeless tobacco products used globally.

Sl. No.	Name of Product	Country/Region of prevalent use	Product composition	Reference
	Afzal	Oman	Tobacco leaves in crushed powder form	Al-Mukhaini et al., 2016
	Chimo	Venezuela	Tobacco, sodium bicarbonate, brown sugar, Mamo´n tree ashes	Stanfill et al., 2011
	Gutkha	India Iran	Mixture of betel leaf, areca nut, slaked lime, catechu, spices, sweet or savory flavorings (aka betel quid) and dried tobacco.	Doherty Lyons et al., 2020
	Iq'mik	Alaska, USA	A homemade mixture of tobacco leaves and tree fungus ash	Patten et al., 2020
	Khaini	India Nepal	Tobacco and slaked lime mixture	Zhao et al., 2021
	Maras	Turkey	Tobacco leaves mixed with the ashes of wood – especially oak, walnut, or grapevine –, in approximate ratios of 1:2 or 1:3.	Taş and Güre, 2020
	Mishri	India	Tobacco roasted on a hot metal plate until it is uniformly black and made in to powder	Ganganahalli et al., 2017
	Moist snuff	USA	Tobacco, flavoring, inorganic salts, humectants	Wang et al., 2021
	Nass	Iran Afghanistan Pakistan	A mixture of tobacco leaves and substances such as lime and ash	Sighaldeh and Charkazi, 2018
	Naswar	Pakistan Iran	A mixture of tobacco, ash and slaked lime added with condiments like green cardamom and mint	Ahmad et al., 2021
	Nuffa/nafha	Northern Africa (Tunisia, Libya, Algeria, Mororcco, Algeria)	Finely powdered tobacco	IARC, 2007
	Qiwam	Bangladesh India	A thick tobacco paste mixed with powdered spices (saffron, cardamom, aniseed and musk)	Sharma et al., 2018
	Rapé	Central and South American countries	Pulverized and finely sifted tobacco leaves mixed with finely ground plant materials (tonka bean, cinnamon, clove buds, etc.) or alkaline ashes	Stanfill et al., 2015
	Sadagura	Assam, India	Sun dried and roasted tobacco leaves with small amounts of black cumin and aniseed seeds as flavoring agents.	Das et al., 2016
	Shammah	Saudi Arabia	Tobacco, slaked lime, black pepper, oils and flavorings	Niaz et al., 2017
	Snuff/snus	Sweden Denmark Finland Norway	Tobacco, moisturizers, sodium carbonate, salt, sweeteners, flavouring	Rygh et al., 2019Hemminki et al., 2021
	Tawa	Ghana	Dried and finely powdered tobacco leaf with some chemicals such as saltpetre (potassium nitrate)	Doku et al., 2010
	Toombak	Sudan	Crushed tobacco leaves with addition of alkaline carbonates, flavourings, and other additives	Sami et al., 2021
	Tuibur	North east India	Tobacco smoke-saturated aqueous concentrate	Madathil et al., 2018
	Zarda	India Bangladesh	Dried and crushed tobacco leaves added with coloring and flavoring agents, chewed by itself, with areca nut or in betel quid	Hrywna et al., 2016

ples, in addition to ^{228Th} in a small number of ST products although it was mentioned that the levels of radionuclides detected may not pose as serious risks to users. The nicotine content of ST products varies widely depending on the type of formulation and many studies have reported as such. Djordjevic and Doran (2009) found that chewing tobacco products contained about 3.4 to 39.7 mg of nicotine per g product, and nicotine levels in dry or moist snuff products ranged from 10.5 to 24.8 and from 4.7 to 24.3 mg/g product, respectively. Sharma et al. (2015) examined nicotine quantity in a variety of ST products, and found that ten different brands of gutka and pan masala contained an average of 6.26 ± 2.65 and 3.25 ± 0.7 mg nicotine/g of tobacco respectively, six different brands of khaini products contained an average of 6.65 ± 2.69 mg nicotine per gram of tobacco, zarda contained an average of 20.35 mg/g, and 5.05 mg/g nicotine in mishri. In another study comparing the nicotine concentration in various ST products, it was found that plain tobacco (2.22 mg/g) had the highest concentration of nicotine followed by ghutka (1.85 mg/g), khaini (0.53 mg/g), pan masala (0.10 mg/g), and supari (0.06 mg/g), respectively (Hegde and Nanukuttan, 2017). Although nicotine content may be comparatively lower in ST products than the smoked form, ST tend to be abused more

Table 2
Summary of the toxic effects elicited by ST on female reproductive physiology.

Observed/Reported effects		References	Model of Study
Ovarian Function	Decreased cell viability and estrogen receptor expression, inhibition of progesterone receptor expression, increases vascular endothelial growth factor expression	Totonchi et al., 2016	Human endometrial stromal primary cells
	Reduced levels of antioxidant defence markers such as Glutathione, glutathione peroxidase, glutathione reductase, and catalase enzymes activity, higher levels of malondialdehyde	Khademi et al., 2019	
	Statistically significant association of tobacco consumption with irregular menstrual cycles	Kulkarni et al., 2018	Rural women aged between 15 years to 49 years
	Inhibition of estradiol production, implications of cytotoxic effects	Sanders et al., 2002	Theca interna and granulosa cells of bovine follicles
	Decrease in estradiol production, mRNA and protein levels of 17β-HSD1 and P450arom	Fan et al., 2019	KGN cell line
	Significant increase in the number of cystic follicles	Singha et al., 2014	Ovarian follicles of non-pregnant mice female
Ovulation and ovarian morphology	Significant reduction in ovary weight, follicle number, survival of superovulated oocyte; reduction of body weight, impairment of ear and eye opening, tooth eruption in F1 offspring	Liu et al., 2019	Premature female mice
	Alteration of healthy to abnormal follicle ratio in ovary, significant reduction in the number of healthy follicles, negative effects on uterine physiological parameters, reduced estrous cycle number	Patil et al., 1999	Female albino rats
	Decline in body weight, ovarian and uterine weight reduction, irregular estrous cycle, substantial histological damage in ovary and uterus	Mandal et al., 2004	Female rats
	Aberrant follicular growth in ovaries due to increased apoptotic cell death	Bordel et al., 2006	Syrian golden hamsters
	Reduction of estrogen level, decrease in germ cell number, deformed organization in ovaries	Al-Mukhaini et al., 2020	Wistar Albino Rats
	Impairment of germ cell development in foetal ovaries, reduction of oocyte number in mother	Cheng et al., 2018	Human fetal ovaries
	Reduction in diameter of zona pellucida-free oocyte, negative alterations in morphology of first polar body	Mai et al., 2014	Female mice
	Premature centromere separation and anaphase, advanced ovarian maturation, reduced proportions of ovulated oocytes	Mailhes et al., 2000	
	Alteration of diestrus phase, disrupted ovarian tissue morphology, increased oxidative stress and DNA damage	Barbhuiya et al., 2020	
Pregnancy Outcomes	Reduced mean hemoglobin (Hb) levels	Subramoney and Gupta, 2008	Adult pregnant women
	Increased risk for 'very and moderately' preterm births	Wikström et al., 2010	
	Higher risks for stillbirths	Baba et al., 2014	
	Significant alterations in ultrastructural characteristics of tertiary villi in low birth-weight group, increased expression of HIF-1α, oxidative DNA damage biomarker	Kumar et al., 2021	
	Micromorphological changes in the placenta leading to loss of functional placentae components	Ashfaq et al., 2008	
	Higher rate (48%) of combined pre-existing and pregnancy-related elevated glucose concentrations	Ratsch et al., 2021	
	Higher incidences of diseases such as cardiovascular, diabetes mellitus, hypertension and spontaneous abortions	Chandirasekar et al., 2019	
	Reduced gestational age at birth and birth weight of newborns	Gupta and Sreevidya, 2004	
	Significant number of stillbirths, reduced birth weights, risk of abnormal delivery	Pratinidhi et al., 2010	
	Increased risk of apnoea in infants born to mothers using ST	De Queiroz Andrade et al. 2020	
	Spontaneous abortion during early pregnancy, stillbirth, preterm delivery, low birth weight, anaemia,	Hossain et al., 2014Ganganahalli et al., 2017Hossain et al., 2018	
	Alterations in heart rate variability, increased low frequency/high frequen ratio	Nordenstam et al., 2017	Infants of snus/snuff users during pregnancy
	Higher systolic blood pressure	Nordenstam et al., 2019	
	Oral cleft formation	Gunnerbeck et al., 2014	
	Significant decrease in birth weight, length, and head circumference	Shenoy et al., 2020	Adult pregnant women with occupational handling of ST
	Significant decrease in gestational period and birth weight, increased implantation problems and neonatal death	Archana et al., 2011	Female mice
	Significant increase in locomotor activity, predisposition to ADHD, reduction in volume and length of cingulate cortex	Zhu et al., 2012	
	Delayed calcification kinetics observed, osteotoxicity	Martinez et al., 2019	Osteogenic human embryonic stem cell

frequently for reasons such as false perceptions of being safer than smoked tobacco, more inconspicuous form of consumption and more widespread restrictions of smoking in public places (Sharma et al., 2015; Siddiqi et al., 2017) and significant levels of nicotine can enter systemic circulation (Saleheen et al., 2014, Li et al., 2018).

3. Smokeless tobacco and ovarian function

The ovaries are complex endocrine glands whose primary functions are production of viable ova for reproduction and steroidogenesis. They are also involved in production of transcription factors, signalling molecules and growth factors that contribute to successful folliculogenesis, fertilization and reproduction (Havelock et al., 2004). Normal ovarian function is under the regulation of other hormones and factors, and it can also be disrupted by various exogenous compounds from lifestyle habits and environmental stressors. These include nicotine from tobacco use and a class of hormone-mimicking compounds known as endocrine disrupting chemicals (Dechanet et al., 2011; Patel et al., 2015; Piazza and Urbanetz, 2019). With the rise in ST consumption amongst women in many parts of the world, it is evident that this trend will be associated with negative reproductive outcomes. Due to the presence of numerous chemicals in ST along with their wide-ranging targets, ST use appears to exert adverse effects on the entire reproductive system in women and at various stages of reproductive function (Willis et al., 2014).

3.1. Endocrine effects

Several studies have suggested that there are significant differences in biological response to nicotine and progression to nicotine dependence between males and females, where females are more sensitive to the effects of nicotine than males probably due to hormonal differences (Sieminska and Jassem, 2014; Chellian et al., 2020; Lee et al., 2021). The primary active ovarian steroid hormones are estradiol and is a key factor in female reproduction, and any dysregulation in its levels can result in certain gynaecological diseases (Fan et al., 2019). Tweed et al. (2012) highlighted the various mechanisms by which tobacco and nicotine could cause systemic disruptions, including modulating physiological factors such as the endocrine system. Totonchi et al. (2016) showed that nicotine, one of the most potent chemicals in tobacco, modulated the expression levels of estrogen receptor, progesterone receptor and vascular endothelial growth factor (VEGF) in endometrial stromal cells in a dose dependent manner that implies a diminishing effect of tobacco on steroid hormone receptors in the gonads. In a community-based study in Karnataka, tobacco use among the women was found to cause significant aberrations in menstrual cycle patterns, with as much as 85% of the participants experiencing dysmenorrhoea and 42% with oligomenorrhoea (Kulkarni et al., 2018). The inhibitory effects of nicotine on the production of prostaglandins, aromatase and hormones such as estrogen and progesterone were also verified *in vitro*, that may be extrapolated to lower endogenous levels in ST users (Bódis et al., 1997; Miceli et al., 2005). Sanders et al. (2002) collected theca interna and granulosa cells from bovine follicles and then cultured them with nicotine or cotinine for 24 h after which culture media were assayed for androstenedione or estradiol levels. Assay results showed that the highest dose of nicotine significantly inhibited production of estradiol in granulosa cells suggesting cytotoxic effects and inhibition of androgen production. Similarly, in KGN cell lines, Fan et al. (2019) demonstrated that nicotine treatment decreased estradiol production as well as mRNA expression of 17β-HSD1 and P450arom, and even at the protein level there was significant reduction of P450arom expression implying the inhibition of estradiol synthesis by ovarian granulosa cells. In histological studies on non-pregnant mice female, ST was shown to significantly increase the number of cystic follicles in the ovary indicating possible adverse effects on granulosa cells (Singha et al., 2014).

3.2. Effects on ovulation and ovarian morphology

Liu et al. (2019) demonstrated that oral administration of 1-(N-methyl-N-nitrosamino)-1-(3-pyridinyl)-4-butanal (NNA), a major component of tobacco-specific nitrosamines to premature female mice resulted in a significant decrease of ovary weight, ovarian follicle number and survival of superovulated oocytes. As a result of increased oxidative stress caused by tobacco use and exposure, Prasad et al. (2016) have also implied multiple adverse effects on oocyte quality, number and reproductive outcome in women. In a study on female albino rats, it was found that nicotine was responsible for altering the ratio of healthy to abnormal follicles in the ovary, with a significant decrease in the number of healthy follicles and uterine physiological parameters were also negatively affected, in addition to reduced estrous cycle number (Patil et al., 1999). In a similar study examining the effects of nicotine, alterations in normal body weight, estrous cycle, and morphological parameters of ovary and uterus were found to be induced by nicotine administration (Mandal et al., 2004). Syrian golden hamsters treated daily with nicotine to mimic regular tobacco consumption showed adverse effects on follicular growth via increased apoptotic cell death in ovaries (Bordel et al., 2006). Mailhes et al. (2000) investigated the effects of nicotine exposure on oocyte maturation *in vivo* in mice ovaries and reported that dosages of 10.0 mg/kg nicotine caused premature centromere separation and premature anaphase, and consequently advanced ovarian maturation in addition to reduced proportions of ovulated oocytes by approximately 50% compared to control groups. Co-exposure of female mice to a type of smokeless tobacco “sadagura” with arsenic which is a common additive of STs resulted in significant changes in diestrus phase, disrupted ovarian tissue morphology, increased oxidative stress and DNA damage in treated groups when compared to control (Barbhuiya *et al.*, 2020). Investigations on the gonadal toxicity of ‘Afzal’, a common type of ST in Oman, found that oral administration of Afzal aqueous extracts to Wistar albino rats resulted in reduced levels of estrogen after the treatment period, and histological examinations revealed a decrease in germ cell number with deformed organization in ovaries (Al-Mukhaini et al., 2020). Cigarette smoke extract, which would be comparable to ST extracts in terms of constituents, fed daily *ad libitum* to mice was shown to cause a reduction in diameter of zona pellucida-free oocyte and perturbations in morphology of first polar body implying the potency of tobacco extracts to decrease the size and quality of oocytes (Mai et al., 2014). Cheng et al. (2018) demonstrated in their study that nicotine *in vitro* could increase apoptosis thereby drastically impairing germ cell development in foetal ovaries and also reduce oocyte number in the mother. Cooper and Moley (2008) reported in their review the possible reasons for delay or absence of conception in some women exposed to tobacco citing that there was a deleterious effect on reproductive functions such as ovarian steroidogenesis, gametogenesis, oocyte maturity, ovulation, oocyte cumulus complex pick-up, gamete and embryo transport by the oviduct, fertilization, and implantation, thereby resulting in infertility. The potential detrimental effects of tobacco use on fertility have also been corroborated by Sahin Ersoy et al. (2017) whose study showed reduced endometrial CXCL12 and FGF2 expression, which are crucial endometrial regulatory cytokines and receptivity markers.

4. Effects on pregnancy outcomes

A considerable number of studies on ST use by the mother during all stages of pregnancy have been consistent in presenting evidence of its adverse effects on both the mother and fetus. Chronic use of ST has direct health implications on the mother, as reported by Subramoney and Gupta (2008) from their population-based cohort study of 918 pregnant women in Mumbai, India, and found that ST use resulted in significantly reduced mean hemoglobin (Hb) levels

(10.00 g/dl) compared with nonusers (10.46 g/dl, $p < 0.000$) which could potentially be associated with anemia. Increased risk for 'very and moderately' preterm births as a result of maternal Swedish snuff use was also reported by Wikström et al. (2010), showing both spontaneous and induced onsets. A study on the associations between maternal snuff use in early pregnancy and risks for stillbirths and early neonatal mortality was conducted in Sweden by Baba et al. (2014) in which 851,371 singleton births were analysed for the purpose. Based on the results, they reported that snuff users in particular and smokers during early pregnancy had higher risks for stillbirths with adjusted odds ratios (ORs) being 1.43 (1.02–1.99) and 1.59 (1.40–1.80), respectively (95% confidence intervals) compared to the non-user group. Conversely, they also found that the similar risks were lower for women who stopped using snuff or stopped smoking before first visit to antenatal care. In one study by Archana et al. (2011), long term exposure of pregnant mice to pan masala, a type of ST product, significantly decreased gestational period and birth weight, in addition to increased implantation problems and neonatal death when compared to control.

A recent secondary data analysis by Shukla et al. (2021) on tobacco use by women showed concerning numbers regarding the prevalence of ST during pregnancy, especially in South-east Asia Region. Since the period from conception up to two years of age in children, i.e., the first 1000 days of life provide a critical stage of development and linear growth, external factors can influence the health conditions of both mother and developing fetus (Quelhas et al., 2018). Recently, Kumar et al. (2021) examined placenta structure collected from tea leave pluckers who are habitual users of ST, and then measured expression levels of hypoxia-inducible factor (HIF)-1α expression and oxidative DNA damage. The sample groups were also divided into low birth weight (LBW) and normal birth weight (NBW) groups after delivery. Their findings showed significant alterations in ultrastructural characteristics of the tertiary villi in LBW group of ST users such as protrusion of endothelial cells into capillary lumen, degenerated nuclei, substantial thickening of trophoblast basement membrane and vasculo-syncytial membrane, aberrations of the microvilli, swollen or damaged mitochondria, and dilatation in endoplasmic reticulum cisternae. They also found substantial decrease in the perimeter, area, and number of the stromal capillary of the tertiary villi of placenta in LBW group when compared to NBW group from the ST users. In addition, when compared with nonusers, ST users showed increased expression of HIF-1α as well as oxidative DNA damage (8-OHdG) biomarker. These findings corroborate similar results by Ashfaq et al. (2008) who reported that wet snuff use was linked to micromorphological changes in the placenta leading to loss of functional placentae components. In a study on Australian Aboriginal women using pituri, a type of ST during pregnancy, results showed that pituri users had a higher rate (48%) of combined pre-existing and pregnancy-related elevated glucose concentrations when compared with smokers (22%) and no-tobacco users (16%). However, surprisingly the lowest rate (14%) of clinically significant post-partum haemorrhage (>1000 ml) was found in pituri chewers compared with 22% of smokers and 36% of the non-users (Ratsch et al., 2021).

Long term consequences of prenatal exposure to gutkha were demonstrated in mice, where the adult male offspring exposed to a high fat diet developed histological evidence of non-alcoholic fatty liver disease in addition to significant alterations in hepatic fibrosis-related cytokines (interleukin (IL)-1b and IL-6) and in hepatic collagen mRNA expression levels compared to control (non-exposed) group (Doherty Lyons et al., 2020). A mouse model for prenatal nicotine exposure from sources such as smoking and ST was used by Zhu et al. (2012) to investigate predisposition of offspring to cognitive disabilities such as deficit hyperactivity disorder (ADHD) and conduct disorder later in life. They concluded from their data that nicotine exposure via oral route significantly increased locomotor activity in both males and female mice and they observed peak activity during the lights-off period of the circadian cycle, i.e., the "active" phase in mice comparable to active phase of ADHD in humans (during the day). They also reported significant reduction in volume of the cingulate cortex (which plays key roles in attention mechanisms) by ~20% and decrease in the length (radial thickness) in the nicotine treated mice.

Similarly, an extensive, systematic review by De Queiroz Andrade et al. (2020) analysing the association between active tobacco use during pregnancy and infant respiratory health suggested that there was a higher risk of apnoea in infants born to mothers using ST throughout their pregnancy. A study conducted amongst the residents of the Western Ghats, Southern India focused on the use of ST such as betel quid, areca nut and tobacco leaf to determine any genotoxic effects. Analysis using genotoxicity biomarkers showed that there was significant DNA damage in tobacco users than non-users, and female ST users in particular reported higher incidences of diseases such as cardiovascular, diabetes mellitus, hypertension and spontaneous abortions (Chandirasekar et al., 2019). In a cohort study of 1217 women in Mumbai, it was found that ST use during pregnancy significantly reduced gestational age at birth as well as birth weight of newborns (Gupta and Subramoney, 2004). The use of 'mishri', another smokeless form of tobacco among pregnant women was found to be responsible for a significantly higher number of stillbirths as well as reduced birth weights in 705 women enrolled for the study, and a relative risk of abnormal delivery of 2.7 was noted for the user group (Pratinidhi et al., 2010). In a prospective observational study, 56 infants of women who used snus regularly during pregnancy were examined for heart rate variability (HRV) and the low frequency and high frequency ratio (LF/HF ratio). Cotinine concentration in urine of the infants were measured and HRV during 2 h of sleep were studied 1–2 months after birth. Higher LF/HF ratio was observed in snus users (mean 3.31; 95% CI 2.78–3.83) compared to control (Nordenstam et al., 2017). Interestingly, Nordenstam et al. (2019) investigated correlation between female snus users throughout their pregnancy and health effects in their children, and found that children of snus users had higher systolic blood pressure than children on non-user control groups. In addition, although both groups had similar heart rates, snus-exposed children showed a greater low/high-frequency ratio, implying altered autonomic cardiac control evidently by nicotine.

A recent study by Martinez et al. (2019) investigated the potential toxic effects of snus extract on live osteogenic human embryonic stem cell cultures since the differentiation process of such cells mimic embryonic development *in vitro*. It was observed that after treatment with 0.1–3% snus extracts, calcification yield of human embryonic stem cells was adversely affected in addition to delayed calcification kinetics observed via video-based calcification analysis, suggesting that from a development perspective in human fetuses, ST use by the mother could result in osteotoxicity during critical stages of development. A cohort study in Bangladesh by Hossain et al. (2014) determined correlative effects of prolonged maternal ST use (>5 years) and pregnancy outcomes. They concluded that concluded that chronic use of ST for longer than 5 years is significantly associated with adverse outcomes of pregnancy such as (i) spontaneous abortion during early pregnancy (ii) stillbirth (iii) preterm delivery (iv) low birth weight of babies. Similar implications on birth weight were observed in a hospital-based cohort study by Ganganahalli et al. (2017) who reported that consumption of 'mishri' by pregnant women led to significant reduction of birth weight and size (about 480 gm lesser and 6.5 cm shorter in birth length) compared to nonusers. Anaemia was also reported in 62% of ST users as well as pre-term delivery in 94.3% compared to non-users. In another study in Bangladesh examining the risk of stillbirth in women consuming ST during pregnancy, it was found that women who used ST daily (>5 times/day) during their first pregnancy had a significantly higher risk of stillbirth when compared to those who used ST less frequently (Hossain et al., 2018). In a case report by Frøisland (2017), a newborn, whose mother had

reported using large amounts of snus (10–20 times/day) during and after pregnancy, showed symptoms of nicotine withdrawal after birth such as continuous crying, sensitivity to light, with high amounts of cotinine in his hair and urine. They also stated that nicotine passes into the breastmilk, suggesting undesirable impacts on the offspring even after birth. Ray et al. (2016) selected maternal ST use during pregnancy as one of two risk factors, along with oral contraceptives (OC), associated with Down syndrome in their offspring, and maternal telomere length were also measured. Mothers and their child with Down Syndrome were selected as test subjects and their results showed that maternal use of ST and OC was significantly linked with higher rates of birthing a child with DS in a case-control analysis. In addition, ST use was also associated with shorter telomere length across all maternal age groups who experienced meiosis I nondisjunction at gametogenesis, indicating potent effects of exogenous chemicals on molecular components of the oocyte in females. A population-based cohort study to determine any possible associations between maternal snuff use and risk of oral cleft formations was conducted by Gunnerbeck et al. (2014) and 975,866 infants that had information on maternal tobacco use were considered for the study, out of which 1761 live births were diagnosed with oral clefts. Results from the analysis showed that the adjusted odds ratios (95% CI) for oral cleft formation in infants of mothers who constantly used snuff throughout their pregnancy was 1.48 [1.00–2.21] when compared to those mothers who stopped using snuff after pregnancy (adjusted odds ratios [95% CI] was 0.71 [0.44–1.14]). In addition to consumption of tobacco, even mere exposure as a result of occupational handling seems to have detrimental effects on pregnant women. In a cohort study in South India on women bidi (a type of hand-rolled cigarette) rollers, it was reported that mean birth weight, length, and head circumference were significantly lower in the exposed group. The risk for 'small for gestational age' was found to be 1.51 (95% CI: 1.12, 2.04; $P = 0.007$) in the exposed group. Serum analysis also detected nicotine (value ≥ 2 ng/mL) in 38.3% of maternal and 29.6% of cord blood in the exposed group with maximum absorption at 125 ng/mL in the maternal and 110 ng/mL in the cord blood (Shenoy et al., 2020).

5. Conclusion

Despite the prevalence of ST consumption across the world, much of the research on pathophysiological outcomes associated with ST use has focused on oral cancer, cardiovascular diseases, respiratory distress and insulin resistance, while detailed studies and literature on the effects on reproductive outcome and gamete quality, especially in women of reproductive age and during pregnancy is still limited. Moreover, the consequences of tobacco exposure via the oral or smokeless route is still largely overlooked, and for reviewing the effects of tobacco constituents such as nicotine, parallels have to be drawn to some extent with smoking. Many investigations on ST-associated reproductive outcomes have also been ambiguous and contradictory for both males and females, and the exact mechanisms of action have not been fully understood. However, majority of the existing literature implicate the use of ST as a risk factor leading to adverse outcomes in pregnancy and reproduction, with long-term health consequences not just for the mother but also for the offspring. The lack of awareness about the dangers of ST products and reluctance towards smoking cessation during pregnancy is an area that still needs intense effort.

There is substantial lack of studies delving into the effects of the constituents of ST products, individually and/or as a whole, on the process of steroidogenesis, reproductive hormones, and their receptors, particularly at the molecular level. Available investigations in both animal models and humans have shown that ST products can negatively affect ovarian morphology, oocyte quantity and quality and derange ovarian steroidogenesis. It is critical to establish the endocrine aspects of reproductive impairments in terms of modulation in the synthesis and levels of circulating hormones like estrogen and testosterone after chronic ST use, since normal reproductive function is quantitatively and qualitatively regulated by such factors. The identification of differentially expressed genes or factors via gonad/gamete transcriptome studies, analysis of their roles and comparative studies of both exposed and non-exposed states at the genetic level are critical in understanding the systemic perturbations that result from ST use. Such studies may contribute new details into the various genetic factors that are affected, their level of susceptibility to exogenous chemicals, their mechanism of action and their role in the molecular cascade leading to reproductive impairment and adverse pregnancy outcomes in women. A few studies have established negative effects of ST exposure on the morphology and micromorphology of the placenta although the mechanisms and long-term consequences not effectively understood. While it is plausible that disruptions in placental growth and differentiation will in turn affect the growth of the fetus, detailed investigations are still required in this regard to better comprehend the impact and etiology of ST use on the interface between mother and fetus during pregnancy.

Several studies in this review have also reinforced the associations between maternal ST use and negative health outcomes in the offspring such as pre-term birth, small for gestational age, low birth weight, cognitive challenges, apnoea and respiratory issues. However, long term studies to monitor and assess the post-natal consequences of these conditions at birth are also still lacking. In addition, since tobacco-induced heritable changes that can be passed on to offspring have been reported, continual evaluations to comprehend the long-term health effects and potential burden of disease in offspring subjected to pre-natal ST exposure would provide a broader perspective into the harms of ST use.

However, studying the health effects of STs will come with its own set of limitations and challenges, since the various ST products can differ greatly in terms of content and bioavailability of its constituents such as nicotine, additives, heavy metals and flavouring agents. There will also undoubtedly be variations in frequency of use and durations of exposure among different populations and cohorts of women. Apart from this, cultural norms and perceptions of such habits can also lead to the stigmatization of ST use, resulting in reluctance to disclose honest patterns of use, and can lead to imprecise interpretations.

The global epidemic of tobacco consumption in smokeless form is still in large parts an understudied issue, especially in terms of reproductive health and outcome in women of reproductive age and during pregnancy. More information is still needed in this regard, to create more awareness about the dangers of ST use in general, to dispel any ambiguity and misinformation and to promote cessation interventions to decrease the burden of preventable illnesses.

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