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Nano-SiO_2 对子代雌性小鼠动情周期的影响 *

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摘要 目的：探讨纳米二氧化硅(Nano-SiO_2)对子代雌性小鼠动情周期的影响。**方法**：选择 8 周龄的 ICR 孕鼠 6 只，随机平均分为两组，一组在 E5.5~E11.5 时隔天注射纳米二氧化硅，另外一组注射 PBS，每组取子代雌性小鼠各 3 只，待其自然生长到 7 周龄时，每日采用巴氏染色法检查其动情周期。**结果**：与注射 PBS 的子代雌性小鼠相比，注射 Nano-SiO_2 的子代雌性小鼠的动情周期的总时间无明显差异(P>0.05)，动情前期的时间明显缩短，动情后期的时间明显延长(P<0.05)，动情期虽然缩短，但差异不显著(P>0.05)。**结论**：孕期暴露于 Nano-SiO_2 未导致子代雌性小鼠动情周期的紊乱，但对其生殖功能可能有一定的影响。

关键词：纳米二氧化硅；动情周期；子代雌鼠

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The Effects of Nano-SiO_2 on the Estrous Cycle of Offspring Female Mice*

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ABSTRACT Objective: To study the effects of nano silica (Nano-SiO_2) on the estrous cycle of female mice. **Methods:** Six ICR pregnant rats at 8 weeks were selected and randomly divided into two groups: one group was injected with nano silica in E5.5-E11.5 every other day, while the other group was injected with PBS. Three offspring female mice were taken daily by PAP staining examination of the estrous cycle in each group when they grow up to 7 weeks were. **Results:** Injection of Nano - SiO_2 of offspring female mice compared with injection of PBS of offspring female mice, there were no significant differences in total time of the estrous cycle of offspring female mice (P>0.05); proestrus time was significantly shorter and metestrus time was significantly prolonged (P<0.05); although the estrous period was shortened, but the difference was not significant (P>0.05). **Conclusion:** Prenatal exposure to Nano-SiO_2 did not result in offspring female mice estrous cycle disorder, but may have a certain influence on the reproductive function.

Key words: Nano-SiO_2; Estrous Cycle; Offspring female mice

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前言

纳米二氧化硅(Nano-SiO_2)是一种非金属氧化物，表面带有不同键合状态的羟基，使纳米二氧化硅具有较高的表面活性，其粒径普遍为 20~60 nm。因 Nano-SiO_2 具有良好的易散性、稳定性和融变性，故具有广泛的用途，是目前我国工业化生产产量最高的一种纳米材料，其产品主要应用于橡胶、生物技术及生物医学等领域[1,2]。由于 Nano-SiO_2 的生物相容性较好，装载量大，性质稳定，因而作为 DNA 和药物载体具有广阔的发展前景。

纳米材料会通过胎盘屏障进入到胚胎体内，对胚胎神经系统、生殖系统产生影响[4-7]，能够影响雄性生殖系统和每日生精量[8-10]。有研究表明，Nano-SiO_2 对生物体具有一定的毒性效应，可引起病变或遗传物质的改变[3]，如对孕鼠尾静脉注射 SiO_2 和 TiO_2 纳米颗粒，会降低子宫重量和增加胎儿再吸收率[11]。关于 Nano-SiO_2 对于小鼠雌性生殖系统的研究主要集中于体外实验及孕期，本研究拟采用腹腔注射 Nano-SiO_2 于孕鼠，观察其对子代雌性小鼠的动情周期的影响。

1 材料与方法

1.1 纳米二氧化硅动物模型的建立

8 周龄的 ICR 孕鼠，在 E5.5~E11.5 时隔天注射纳米二氧化硅，另外一组注射 PBS，每组分别 3 只孕鼠。待小鼠出生后，让其自然生长，子代小鼠到 7 周龄时，体重为 28~34g，每一组取子代雌性小鼠各 3 只。

1.2 纳米二氧化硅的制备

利用溶胶 - 凝胶法制备单分散球形的纳米二氧化硅，以 TEOS 为硅源，氨作为催化剂[12]，乙醇作溶剂可制备分散颗粒[13]，控制氨和水的浓度、水解温度，使最后合成的纳米二氧化硅的粒径在 30～50 nm。

1.3 小鼠动情周期的观察

采用巴氏染色法检查动情周期，每天早上 9 点开始取样，

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通过观察细胞形态变化判断小鼠动情周期变化。方法:用棉签蘸取生理盐水,轻轻插入生殖道少许,旋转一圈取出,涂抹在载玻片上,用 95%的乙酸乙醇固定,固定 15 分钟后,用巴氏染液进行染色,待涂片干燥后显微镜下观察,连续观察 30 天。

巴氏染色法检测原理:细胞中的细胞核是酸性物质组成的,因而易与碱性染料作用;而细胞质含有碱性物质,因而易与酸性染料作用。巴氏染液利用这一特性对细胞进行多色性染色。实验观察指标主要是通过阴道脱落细胞的变化来判定动情周期[14](见表 1)。

表 1 大鼠动情周期的细胞形态学观察

Table 1 Cell morphological characteristics at every stage of the estrous cycle

阶段 Stage	持续时间(d) Time(d)	细胞形态学观察 Cell morphological characteristics
动情前期 Proestrus	0.7-0.9	全部是有核上皮细胞,偶可见少量角化细胞 All nucleated epithelial cells, a small amount of keratinocytes
动情期 Estrus	0.4-0.6	全部是角化细胞,偶可见少量有核上皮细胞 All keratinocytes, a small amount of nucleated epithelial cells
动情后期 Metaestrus	0.4-0.6	白细胞、角化细胞、有核上皮细胞均可见 White blood cells, keratinocytes, nucleated epithelial cells
动情间期 Diestrus	2.5-2.9	大量白细胞及少量有核上皮细胞或粘液 A large number of white blood cells and a small amount of nucleated epithelial cells or mucilage

1.4 统计分析

实验数据以均数± 标准差($\bar{x}$± s)表示,实验数据采用 EXCEL 进行统计分析,注射纳米二氧化硅与注射 PBS 的子代雌性小鼠的动情周期的时间相比较用 t-test。

2 结果

2.1 Nano-SiO_2 对子代雌性小鼠动情周期的影响

采用巴氏染色法检查子代雌性小鼠的动情周期,通过观察细胞形态变化判断小鼠动情周期变化,结果如图 1 显示。

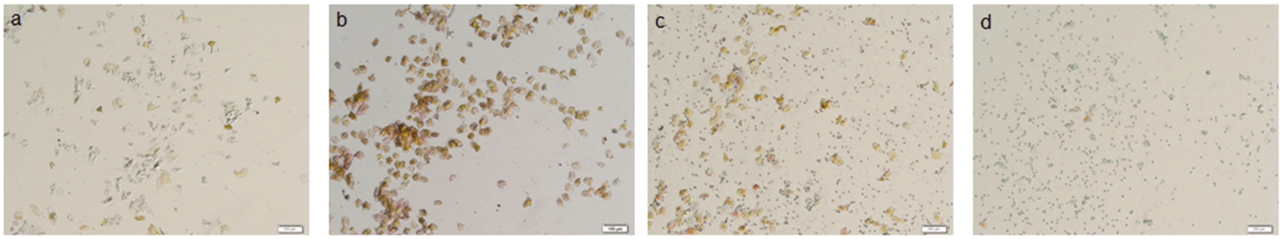

图 1 雌性小鼠动情周期各时相的细胞形态特征

Fig.1 Cell morphological characteristics at every stage of the estrous cycle

注:a) 动情前期;b) 动情期;c) 动情后期;d) 动情间期

Note:a) Proestrus; b) Estrus; c) Metaestrus; d) Diestrus

根据动情期的各个时相的细胞形态特征,可知注射 Nano-SiO_2 和 PBS 的子代雌性小鼠的平均动情周期分别为(4.96± 0.44)和(4.48± 0.18)天,无明显差异(P>0.05)。

2.2 Nano-SiO_2 对小鼠动情周期各时相的影响

表 3 所示为动情周期各时相的天数,结果显示:与注射 PBS 的子代雌性小鼠相比,注射 Nano-SiO_2 的子代雌性小鼠动情前期的时间明显缩短,动情后期的时间明显延长(P<0.05),而动情期虽然缩短,但差异不显著(P>0.05)。

表 2 注射 Nano-SiO_2 与注射 PBS 的子代雌性小鼠的平均动情周期的比较($\bar{x}$± s)

Table 2 Comparison of the average estrus cycle between the mice offspring injected by Nano-SiO_2 and PBS($\bar{x}$± s)

Group	Number(n)	Estrus cycle(d)
Nano-SiO_2	9	4.96± 0.44
PBS	9	4.48± 0.18

表 3 注射 Nano-SiO_2 与注射 PBS 的子代雌性小鼠动情周期各时相天数的比较($\bar{x}$± s)

Table 3 Comparison of the every stage of estrus cycle between the mice offspring injected by Nano-SiO_2 and PBS($\bar{x}$± s)

Group	Proestrus(d)	Estrus(d)	Metaestrus(d)	Diestrus(d)
Nano-SiO2	0.82± 0.15*	1.24± 0.17	1.82± 0.19*	1.07± 0.41
PBS	1.16± 0.23	1.43± 0.29	1.02± 0.32	0.87± 0.31

Note:*P<0.05 Compared with PBS.

3 讨论

随着纳米材料的广泛应用，在人们关注其优越性的同时，纳米材料的对生物体和环境是否会造成潜在危害也受到了越来越多学者们的关注。有研究表明，磷酸钙纳米粒子会影响体外培养的人卵巢颗粒细胞的细胞周期并导致其凋亡[15]；金纳米颗粒能够增加体外培养的人卵巢颗粒细胞雌激素的分泌量[16]；纳米颗粒在 E11.5 天之前可通过胎盘屏障进入到胚胎体内，并造成一定的影响[17-19]。这些研究结果使人们开始关注于纳米材料对雌性生殖的安全性。

检测雌性动物动情周期的变化是一种评估雌性生殖健康相对简单且可靠的方法[20]。在雌性成鼠的非妊娠生殖活动中，动情状态呈周期性变化，生殖卵子的形成和性激素的分泌也都呈周期性波动，这种周期的变化是由下丘脑 - 垂体 - 性腺轴周期变化所致。机体下丘脑 - 垂体 - 性腺轴平衡调节功能的正常与否是维持其正常雌性性腺生殖功能的重要保证[21]，动情周期则是性腺生殖功能正常与否的直接标志。在卵巢表现的周期性排卵和其分泌的雌性激素水平周期性变化的同时，其阴道细胞也呈现周期性变化，因而可通过阴道细胞的周期性变化判断小鼠的动情周期。小鼠的动情周期分为动情前期、动情期、动情后期、动情间期。孕期暴露于纳米颗粒会导致出生后的小鼠生殖功能和内分泌系统的破坏[22,23]。动情周期的紊乱是反应性激素失调的敏感指标[24]，也是评价外源化合物对内分泌影响的特征指标。Omura[25]用两代繁殖实验研究三丁基锡对雌性大鼠的生殖毒性，结果发现第二代雌性大鼠阴道张开延迟，表明其动情周期不规则。因此，动情周期异常是雌性动物受到外源化合物作用后的早期反映。

动情前期和动情期是卵巢排卵期，动情后期是分泌孕酮及黄体形成的时期。本研究通过观察注射 Nano-SiO_2 的子代小鼠的动情周期并分期各期时间的变化，发现注射 Nano-SiO_2 的子代小鼠并没有发生动情周期紊乱的现象，但其动情前期明显缩短，表明 Nano-SiO_2 在一定条件下有抑制排卵的作用，可能用于避孕；其动情后期显著延长，表明 Nano-SiO_2 可能影响黄体的形成和功能的发挥，有可能导致流产；但其动情期无显著变化。由此可见，Nano-SiO_2 对子代小鼠的生殖系统有一定的影响。

目前，纳米材料对雌性生殖发育的影响及安全性仍然是纳米毒理学高度关注的领域。在后续的研究中，我们将进一步用注射 Nano-SiO_2 的子代雌鼠与正常雄鼠交配，检测其生殖能力的变化，必要时对生殖相关激素(LH、FSH 等)进行动态检测，以期深入探讨 Nano-SiO_2 对雌性生殖的安全性。

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