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Reproduction, Fertility and Development

Supplementary Material

Differential effect of melatonin on ram spermatozoa depending on the allelic variant of the *RsaI* polymorphism of the *MTR1A* gene, incubation medium and season

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Supplemental Tables

Supplemental Table 1. Percentages of total motile spermatozoa in TALP-incubated sperm samples (3h at 39 °C, 100% humidity, 5% CO_2) from rams carrying different *Rsa*I genotypes without (Control, TALP) or with melatonin at 1 µM (TALP + Mel 1 µM) during the non-reproductive and the reproductive season. Data are shown as median, first and third quartile (n=11 for non-reproductive season and n = 10 for reproductive season).

Parameter	Genotype	Non-reproductive season			Reproductive season		
		Control (TALP)	TALP + Mel 1 µM	*P* value	Control (TALP)	TALP + Mel 1 µM	*P* value
Total motile sperm	CC	90.31 (86.67, 91.68)	85.70 (81.26, 90.36)	0.1289	56.57 (32.00, 75.11)	66.41 (41.88, 79.51)	0.1602
	CT	85.33 (79.32, 87.97)	87.39 (76.10, 91.27)	0.7695	64.83 (59.03, 75.11)	63.18 (53.57, 70.70)	0.4316
	TT	89.47 (85.25, 91.38)	88.24 (86.05, 91.71)	0.8203	71.39 (51.63, 89.31)	65.34 (37.19, 84.58)	0.4922

Supplemental Table 2. Percentages of viable, viable with low levels of ROS, viable with low levels of superoxide ($O_2^{\cdot-}$) and viable with active mitochondria spermatozoa in TALP-incubated sperm samples (3h at 39 °C, 100% humidity, 5% CO_2) from rams carrying different *Rsa*I genotypes without (Control, TALP) or with melatonin at 1 µM (TALP + Mel 1 µM) during the non-reproductive and the reproductive season. Data are shown as median, first and third quartile (n=11 for non-reproductive season and n = 10 for reproductive season).

Parameter	Genotype	Non-reproductive season			Reproductive season		
		Control (TALP)	TALP + Mel 1 µM	*P* value	Control (TALP)	TALP + Mel 1 µM	*P* value
Viable sperm (%, CFDA+/PI-)	CC	77.70 (72.90, 83.05)	75.95 (68.60, 84.93)	0.4688	59.60 (53.80, 73.30)	68.20 (52.93, 72.28)	0.5469
	CT	69.55 (63.93, 74.78)	76.55 (67.23, 82.23)	0.1484	71.10 (59.15, 72.75)	71.80 (67.75, 75.00)	0.3203
	TT	77.20 (72.20, 85.05)	79.30 (75.60, 84.20)	0.3438	68.05 (58.55, 74.48)	68.00 (53.40, 71.15)	0.0781
Viable sperm with low levels of ROS (%, H_2DCFDA-/PI-)	CC	73.35 (64.78, 81.60)	68.60 (57.90, 80.05)	0.4609	59.30 (37.10, 68.90)	68.20 (57.60, 68.70)	0.4375
	CT	73.48 69.33, 75.75)	70.50 (55.25, 75.10)	0.4688	68.30 (61.75, 68.60)	67.30 (57.00, 68.45)	0.4453
	TT	76.35 (66.30, 83.60)	76.30 (74.08, 82.85)	0.5781	60.00 (54.30, 69.25)	59.40 (56.70, 69.85)	0.9102
Viable sperm with low levels of superoxide (%, E-/Yo-Pro1-)	CC	52.60 (36.70, 66.00)	61.45 (41.08, 65.68)	0.8438	49.10 (44.28, 51.50)	49.10 (43.58, 54.08)	0.8438
	CT	58.90 (52.30, 66.30)	52.25 (41.25, 66.15)	0.2500	52.10 (42.00, 67.30)	54.50 (41.65, 57.60)	0.4961
	TT	61.45 (57.95, 67.18)	59.90 (44.55, 63.65)	0.2500	45.30 (39.65, 56.30)	40.70 (35.00, 51.75)	0.3594
Viable sperm with active mitochondria (%, Mitotracker® Deep Red FM+/ Yo-Pro-1-)	CC	72.80 (63.20, 77.70)	71.70 (66.68, 74.30)	0.2969	61.15 (48.78, 66.10)	61.30 (56.88, 67.75)	0.5469
	CT	70.10 (65.60, 77.80)	71.40 (66.90, 79.70)	0.3008	63.10 (60.10, 67.05)	62.60 (60.55, 67.75)	0.9102
	TT	66.40 (37.30, 73.00)	66.00 (39.40, 73.80)	0.1992	55.80 (47.85, 60.85)	50.90 (40.50, 62.35)	0.9102

Supplemental Table 3. Percentages of viable with no active caspases spermatozoa in TALP-incubated sperm samples (3h at 39 °C, 100% humidity, 5% CO_2) from rams carrying different *Rsa*I genotypes without (Control, TALP) or with melatonin at 1 µM (TALP + Mel 1 µM) during the non-reproductive and the reproductive season. Data are shown as median, first and third quartile (n=11 for non-reproductive season and n = 10 for reproductive season).

Parameter	Genotype	Non-reproductive season			Reproductive season		
		Control (TALP)	TALP + Mel 1 µM	*P* value	Control (TALP)	TALP + Mel 1 µM	*P* value
Caspase activation (%, FITC-VADFMK-/EthD-1)	CC	56.00 (33.00, 65.90)	50.70 (45.10, 63.50)	0.2061	28.95 (24.33, 46.03)	35.80 (20.45, 49.45)	0.5703
	CT	51.20 (33.61, 54.10)	66.90 (29.75, 71.35)	0.4609	42.95 (26.03, 55.80)	39.60 (22.10, 48.60)	0.6953
	TT	57.90 (51.10, 64.90)	66.00 (39.40, 73.80)	0.1230	41.70 (32.93, 53.00)	42.00 (22.70, 58.48)	0.4316

Supplemental Table 4. Percentages of total motile spermatozoa in incubated sperm samples (3h at 39 °C, 100% humidity, 5% CO_2) in TALP medium with cAMP-elevating agents (CK medium) from rams carrying different *Rsa*I genotypes without (Control, CK) or with melatonin at 1 µM (CK + Mel 1 µM) during the non-reproductive and the reproductive season. Data are shown as median, first and third quartile (n=11 for Non-reproductive season and n = 10 for Reproductive season).

Parameter	Genotype	Non-reproductive season			Reproductive season		
		Control (CK)	CK + Mel 1 µM	*P* value	Control (CK)	CK + Mel 1 µM	*P* value
Total motile sperm	CC	70.45 (34.82, 85.27)	63.88 (46.30, 86.40)	0.4961	63.71 (50.14, 76.47)	54.79 (39.04, 76.73)	0.3828
	CT	67.15 (46.29, 77.74)	61.19 (35.69, 85.12)	0.6523	68.82 (50.02, 73.47)	49.86 (31.06, 74.12)	0.2031
	TT	80.67 (63.04, 84.81)	74.87 (54.83, 87.14)	0.9999	54.59 (45.49, 84.42)	42.03 (36.00, 67.65)	0.1953

Supplemental Table 5. Percentages of viable, viable with low levels of ROS, viable with low levels of superoxide ($O_2^{\cdot-}$) and viable with active mitochondria spermatozoa in sperm samples incubated in TALP medium with cAMP-elevating agents (3h at 39 °C, 100% humidity, 5% CO_2) from rams carrying different *Rsa*I genotypes without (Control, CK) or with melatonin at 1 µM (CK + Mel 1 µM) during the non-reproductive and the reproductive season. Data are shown as median, first and third quartile (n=11 for non-reproductive season and n = 10 for reproductive season).

Parameter	Genotype	Non-reproductive season			Reproductive season		
		Control (CK)	CK + Mel 1 µM	*P* value	Control (CK)	CK + Mel 1 µM	*P* value
Membrane integrity (%, CFDA+/PI-)	CC	77.45 (67.30, 79.63)	76.20 (67.70, 77.80)	0.5781	74.75 (68.25, 77.50)	74.95 (71.20, 76.78)	0.9766
	CT	72.20 64.88, 79.28)	66.45 (57.55, 75.10)	0.2109	71.50 (69.53, 75.00)	72.90 (68.15, 75.23)	0.7695
	TT	74.90 (72.85, 75.83)	76.95 (74.03, 77.43)	0.1094	74.80 (71.45, 79.10)	75.50 (70.45, 79.93)	0.7109
Intracellular superoxide ($O_2^{\cdot-}$) levels (%, E-/Yo-Pro1-)	CC	65.10 (53.40, 70.90)	61.20 (50.60, 66.45)	0.8125	51.50 (35.40, 59.85)	50.20 (33.45, 53.20)	0.6953
	CT	55.60 (43.73, 62.26)	56.15 (41.10, 64.00)	0.9453	51.30 (38.08, 58.53)	53.55 (39.88, 58.15)	0.6074
	TT	61.30 (38.30, 69.20)	63.20 (61.20, 67.40)	0.9375	57.75 (34.90, 65.58)	58.00 (46.78, 60.95)	0.7695

Supplemental Table 6. Percentages of viable without PS inversion spermatozoa in sperm samples incubated (3h at 39 °C, 100% humidity, 5% CO_2) in TALP medium with cAMP-elevating agents (CK medium) from rams carrying different *Rsa*I genotypes without (Control, CK) or with melatonin at 1 µM (CK + Mel 1 µM) during the non-reproductive and the reproductive season. Data are shown as median, first and third quartile (n=11 for non-reproductive season and n = 10 for reproductive season).

Parameter	Genotype	Non-reproductive season			Reproductive season		
		Control (CK)	CK + Mel 1 µM	*P* value	Control (CK)	CK + Mel 1 µM	*P* value
PS inversion (Annexin V-/ IP-)	CC	69.75 (38.83, 72.53)	71.30 (32.88, 74.40)	0.8457	61.85 (49.60, 73.85)	65.95 (48.93, 73.83)	0.2324
	CT	58.50 (42.90, 74.05)	62.10 (31.08, 71.95)	0.7539	64.60 (57.23, 75.85)	63.60 (51.28, 69.63)	0.4316
	TT	73.75 (64.03, 75.33)	75.25 (57.13, 77.75)	0.9102	68.55 (57.55, 75.85)	63.15 (59.18, 69.80)	0.3105