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Increased Progesterone Production in Cumulus–Oocyte Complexes of Female Mice Sired by Males With the Y-Chromosome Long Arm Deletion and its Potential Influence on Fertilization Efficiency

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Abstract

It was revealed previously that B10.BR(Y^{del}) females sired by males with the Y-chromosome long arm deletion differ from genetically identical B10.BR females sired by males with the intact Y chromosome. This is interpreted as a result of different epigenetic information which females of both groups inherit from their fathers. In the following study, we show that cumulus–oocyte complexes ovulated by B10.BR(Y^{del}) females synthesize increased amounts of progesterone, which is important sperm stimulator. Because their extracellular matrix is excessively firm, the increased progesterone secretion belongs presumably to factors that compensate this feature enabling unchanged fertilization ratios. Described compensatory mechanism can act only on sperm of high quality, presenting proper receptors. Indeed, low proportion of sperm of Y^{del} males that poorly fertilize B10.BR(Y^{del}) oocytes demonstrates positive staining of membrane progesterone receptors. This proportion is significantly higher for sperm of control males that fertilize B10.BR(Y^{del}) and B10.BR oocytes with the same efficiency.

Keywords

cumulus cells, progesterone, spermatozoa, steroidogenesis, Y chromosome

Introduction

In the mouse, the male-specific region of the Y-chromosome long arm (MSYq) is composed of highly repetitive DNA sequences that play a crucial role in sperm development (spermiogenesis). Four multicopy gene families: *Ssty1/2*, *Sly*, *Asty*, and *Orly* have been identified in this region.[1-3] Most information about their function derives from analysis of mutant male mice, which are charged with various deletions in the long arm of the Y chromosome.[2] Such analysis enrich the knowledge about contribution of the Y chromosome to the processes associated with male fertility, also in people. Deletions in the long arm of the Y chromosome affect many men with sperm dysfunctions and fertility disorders.[4,5]

B10.BR and B10.BR-Y^{del} congenic strains of mice belong to very useful experimental models for studies on MSYq genes. Animals of both strains have the same genotypes, but while B10.BR males have an intact Y chromosome, B10.BR-Y^{del} males carry the Y chromosome with a large deletion covering approximately two-third of the long arm.[6] Despite the loss of the great part of MSYq sequences, B10.BR-Y^{del} males are fertile, but they produce spermatozoa with definitely lower quality than control B10.BR males.[6-11] Additionally, in the offspring of mutant males, a characteristic sex ratio skew in favor of females is observed, which does not occur in the offspring of control males (published data,[1,12] breeding records of the authors' laboratory). Experiments on XY^{RIII}qdel male mice (with a very similar to B10.BR-Y^{del} deletion removing 2/3 of the MSYq) proved that this phenomenon is caused not by a reduced frequency of Y-bearing sperm in ejaculate but by their poorer fertilizing ability in comparison with X-bearing counterparts.[13] Genetically identical females of the both congenic strains vary in some reproductive features as well,[14-16] which

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can be explained only by transgenerational influence of parental Y chromosomes. Epigenetic modifications of genetic material in X-bearing spermatozoa seem to be the possible mechanism of such influence. We hypothesize that genes of the long arm of the Y chromosome are engaged in the establishment of epigenetic marks in male germ cells, and the partial loss of Yq sequences in mutant males affects the regulation of this complicated process. Altered epigenetic information may be inherited by female progeny modulating some of its qualities.[15,16]

The involvement of the Y chromosome into sperm epigenetics has been already demonstrated in many studies. The comparison of sex-specific methylation patterns in germ cells of sex-reversed and control mouse embryos revealed a clear methylating activity dependent on the presence of the Y chromosome and autonomous from the effect of gonadal environment. This activity is not associated with *Sry* expression, because it was observed in cells with the *Sry*-deleted Y chromosome.[17] In spermatids of mice with partial and entire deletions of the male-specific Yq content, increased transcription from both sex chromosomes and various histone code abnormalities were discovered, which imply that MSYq genes are engaged in postmeiotic sex chromosome repression accompanied by chromatin remodeling.[18] Construction of transgenic shSLY mice allowed to prove that multicopy *Sly* (*Sycp3-like Y-linked*) gene plays the predominant role in these processes,[19] although contribution of other genes cannot be excluded. Comet assays, which showed the dramatic increase in DNA damage in sperm of mice with a severe Yq deletion, confirmed the significance of MSYq genes for the correct chromatin remodeling and thus for maintaining DNA integrity during spermiogenesis.[20]

As mentioned earlier, our previous experiments have revealed some phenotypic differences between female mice sired by B10.BR-Y^{del} and B10.BR males. We named the first group of females B10.BR(Y^{del}) to distinguish them from genetically identical females of the control B10.BR strain. Extracellular matrix of cumuli oophori surrounding ovulated oocytes of B10.BR(Y^{del}) females is more abundant with proteins than matrix of cumuli oophori surrounding oocytes of B10.BR females.[16] It is also more resistant to enzymatic treatment as well as to sperm activity in vitro.[14,15] These features decrease fertilization efficiency of B10.BR(Y^{del}) oocytes, but only in the case of mating with B10.BR-Y^{del} males, producing poor-quality sperm.[15] Additionally, females sired by males with the deleted Y chromosome exhibit increased prostaglandin E_2-EP2 receptor signaling in their periovulatory processes.[16] Apart from many other important functions, this signaling stimulates progesterone synthesis in cumulus granulosa cells.[21,22] In the present study, we compared progesterone production in cumulus–oocyte complexes (COCs) ovulated by B10.BR and B10.BR(Y^{del}) females. We assessed expression levels of *Cyp11a1* gene (encoding cholesterol side-chain cleavage cytochrome P450, first enzyme on steroid biosynthetic pathway converting cholesterol to pregnenolone) as well as *Hsd3b1* gene (encoding 3β-hydroxysteroid dehydrogenase, enzyme transforming pregnenolone to progesterone). Additionally, we measured messenger RNA (mRNA) level of *Cyp19a1* gene encoding P450 aromatase responsible for conversion of testosterone into 17β-estradiol.[23,24] This is proved that although COCs synthesize predominantly progesterone after ovulation, small amounts of estradiol are also secreted at that time.[25-28] Next, we measured progesterone accumulation in the medium where B10.BR and B10.BR(Y^{del}) COCs were incubated.

In the female genital tract, progesterone secreted by cumulus cells is sperm chemoattractant.[29-31] It also stimulates capacitation, hyperactivated motility, and acrosomal reaction of spermatozoa.[32-34] All these effects of progesterone support successful fertilization. Progesterone interacts with specific sperm membrane receptors, activating nongenomic second messenger-mediated pathways.[32,33,35] Biochemical signals derived from cumulus cells can be recognized only by competent spermatozoa, presenting proper receptors on their surfaces. For that reason at the end of our work, we assessed availability of progesterone receptors on spermatozoa produced by males with the normal (B10.BR) and with the deleted (B10.BR-Y^{del}) Y chromosomes.

Materials and Methods

Animals and Hormonal Treatment

Adult, 10- to 12-week-old female and male mice from the 2 congenic B10.BR/SgSn and B10.BR-Y^{del} strains were used in the experiments. Males from the mutant B10.BR-Y^{del} strain are characterized by a partial deletion in the long arm of the Y chromosome. Genetically, identical males from the control B10.BR strain have the intact Y chromosome. To avoid genetic divergence between the 2 congenic strains, in each generation B10.BR-Y^{del} males are backcrossed to B10.BR females. Females sired by males with the normal and with the deleted Y chromosomes have the same genotype. To distinguish these 2 experimental groups of females, we named them as B10.BR and B10.BR(Y^{del}), respectively.

Mice were maintained under a 12-hour light–dark cycle and fed on a commercial laboratory diet. To stimulate superovulation, female mice were injected intraperitoneally with 7 IU of pregnant mare serum gonadotrophin (Inter Vet, Holland) and 48 hours later with 7 IU of human chorionic gonadotrophin (hCG; Inter Vet). The experiments were performed in accordance with Polish legal requirements, under the license of the First Local Ethics Commission for Animal Experiments at the Jagiellonian University.

RNA Isolation From Cumulus Cells and Real-Time Reverse Transcriptase-Polymerase Chain Reaction

The B10.BR and B10.BR(Y^{del}) female mice were killed by cervical dislocation, 14 hours after hCG injection. The COCs collected from both oviducts of each female were dispersed with hyaluronidase (Sigma-Aldrich, Germany) dissolved in

phosphate-buffered saline (PBS; 50 IU/mL). The oocytes were removed using thin capillaries. The remaining cumulus cells were pelleted by gentle centrifugation (300*g*, 10 minutes, 20°C) and immediately subjected to RNA isolation with the Nucleo Spin II kit (Macherey-Nagel, Germany). Integrity of the obtained RNA was confirmed by gel electrophoresis. RNA purity and concentration were assessed with Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham). Total RNA of 0.5 μg of each sample was reverse transcribed into complementary DNA (cDNA) using the High-Capacity cDNA Reverse Transcription Kit with random hexamers (Applied Biosystems, Foster City, California). Real-time polymerase chain reactions (PCRs) were run in a final volume of 15 μL on StepOne-Plus Real-Time PCR System (Applied Biosystems) using 96-well optical plates (Applied Biosystems). The reaction mixtures included 2 μL of cDNA sample (diluted 1:2), RNase-free water (Sigma-Aldrich), TaqMan Gene Expression Master Mix (Applied Biosystems), and TaqMan Gene Expression Assay (Applied Biosystems; *Cyp11a1*: Mm00490735_m1, *Hsd3b1*: Mm01261921_mH, *Cyp19a1*: Mm00484049_m1, *Actb*: Mm00607939_s1). Polymerase chain reactions were incubated at 95°C for 10 minutes followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. β-Actin (*Actb*) was used as the endogenous reference gene. All reactions were run in triplicate, and average threshold cycle (Ct) was calculated. For each sample, the Ct value of the endogenous reference gene was subtracted from the Ct values of the target genes to obtain ΔCt values and to normalize the PCRs for the amount of cDNA added to the subsequent reactions. For graphical presentation and statistical analysis, relative mRNA level indexes of *Cyp11a1*, *Hsd3b1*, and *Cyp19a1* genes were generated with $2^{-\Delta Ct}$ formulae.[36]

Culture of COCs

The B10.BR and B10.BR(Y^{del}) females were killed at 14 hours post-hCG injection. Cumulus–oocyte complexes isolated from both oviducts of each female were counted and transferred into humidified cell culture dish (Corning Inc, New York) containing 1 mL of Eagle minimum essential medium with Earle salts, L-glutamine, and sodium bicarbonate (Sigma-Aldrich) supplemented with 5% heat-inactivated fetal bovine serum (Sigma-Aldrich). After 4.5 hours incubation (37°C, 5% CO_2), culture medium was separated from the COCs by gentle centrifugation (300*g*, 10 minutes, 20°C) and frozen at −20°C until further investigation. Groups counting less than 20 COCs were excluded from the experiment.

Radioimmunoassay

Steroid concentrations in culture media were estimated by specific radioimmunoassays as previously described.[37] Progesterone levels were determined using (1,2,6,7-^{3}H)progesterone (specific activity 96 Ci/mmol; GE Healthcare, Amersham International, United Kingdom) as a tracer and an antibody induced in sheep against 11α-hydroxyprogesterone succinyl–bovine serum albumin (BSA; a gift from Professor B. Cook, University of Glasgow, Scotland). Cross-reactions were 1.8% with pregnenolone, 1.5% with corticosterone, 0.8% with 17α-hydroxyprogesterone, and 0.1% with testosterone. Binding of other steroids was below 0.01%. The lower level of sensitivity of the assays was 20 pg. Coefficients of variation within and between assays were below 5.0% and 9.8%, respectively.

Estradiol concentrations were measured using (2,4,6,7-^{3}H) estradiol (specific activity 88 Ci/mmol; GE Healthcare, Amersham International) as a tracer and rabbit antibody against estradiol-17β-6-carboxymethyloxime–BSA (a gift from Prof R. Rembiesa, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland). Cross-reactions were 1% with 16-keto-estradiol-17β, 0.8% with estrone, 0.8% with estriol, 0.1% with testosterone, and less than 0.1% with other steroids. The lower level of sensitivity was 5 pg. Coefficients of variation within and between assays were below 4% and 7.5%, respectively. All samples were assayed in duplicate. The results obtained for subsequent incubations were recalculated per single COC.

Detection of Surface Progesterone Receptors in Spermatozoa

Sperm preparation was performed using human tubal fluid (HTF) medium[38] containing 101.6 mmol/L NaCl, 4.69 mmol/L KCl, 0.37 mmol/L KH_2PO_4, 2.04 mmol/L $CaCl_2$, 0.2 mmol/L $MgSO_4$, 25 mmol/L $NaHCO_3$, 2.78 mmol/L glucose, 0.33 mmol/L Na pyruvate, 21.4 mmol/L Na lactate, penicillin 100 U/mL, streptomycin sulfate 50 μg/mL, and phenol red 10 μg/mL (all chemicals obtained from Sigma-Aldrich). Sperm were collected from both cauda epididymides of B10.BR and B10.BR-Y^{del} males and preincubated for 0.5 hours (37°C, 5% CO_2) in 1 mL of HTF medium supplemented with 4 mg of BSA (Sigma-Aldrich; cat. no A-6003).

After centrifugation (300*g*, 10 minutes, 25°C) and removal of BSA-containing supernatant, the sperm pellet was resuspended in 2 mL of HTF medium and loaded onto a discontinuous gradient of 45% and 90% Percoll (Pharmacia, Sweden) in HTF medium. The gradient was centrifuged (560*g*, 20 minutes, 25°C), allowing motile sperm to sediment as a pellet at the bottom of conical tube. The top 2 layers (containing debris, nonsperm cells, and immotile sperm) were removed together with Percoll solutions. The bottom motile sperm pellet was washed in HTF (300*g*, 10 minutes, 25°C) and then capacitated in 1 mL of HTF with BSA (1 hour, 37°C, 5% CO_2). After next centrifugation (300*g*, 10 minutes, 25°C) and supernatant removal, spermatozoa were suspended in PBS without calcium and magnesium (Biomed-Lublin, Poland) at a concentration of 1×10^6 cells/mL.

Progesterone 3-(o-carboxymethyl)oxime–BSA-fluorescein isothiocyanate conjugate (P-BSA-FITC; Sigma-Aldrich) was dissolved in PBS at a concentration of 200 μg/mL and treated with dextran-coated charcoal (Sigma-Aldrich) to remove nonconjugated progesterone.[39] Sperm suspension of 25 μL was added to 75 μL of P-BSA-FITC solution,

mixed gently, and incubated overnight at 4°C in the dark. As a negative control, another sperm sample was incubated at the same conditions in BSA–FITC solution (Sigma-Aldrich). After incubation, the cells were washed with PBS, centrifuged (300*g*, 10 minutes, 4°C), and resuspended in PBS. Sperm suspension of 10 μL was smeared onto a glass slide, stained for 5 minutes with 4′,6-diamidino-2-phenylindole dihydrochloride (Sigma-Aldrich; diluted 1:2000 in PBS), mounted with Citifluor AF1 medium (Citifluor, United Kingdom), covered with a coverslip, and sealed with nail polish. The preparations were examined with Nikon Eclipse TS100 microscope (Nikon Instruments Inc, Japan) equipped with epifluorescence attachment. For each preparation, 200 successive spermatozoa were classified as positive or negative for P-BSA-FITC staining.

Statistical Analyses

Statistical analyses of gene expression levels were performed on $2^{-\Delta Ct}$ values. The results obtained in percentages were normalized using angular transformation (arcsin). Differences between groups were evaluated with the Student *t* test (for equal variances). Significance was assumed at $P < .05$.

Results

Expression of all examined genes: *Cyp11a1*, *Hsd3b1*, and *Cyp19a1* was detected in cumulus cells surrounding ovulated oocytes of B10.BR and B10.BR(Y^{del}) females (Figure 1). *Cyp11a1* gene is characterized with the highest expression, persisting on the same level in cells of the both groups of females ($P = .5$). Relatively high expression is also typical for *Hsd3b1* gene which is, however, significantly more active in B10.BR(Y^{del}) cumulus cells in comparison with control B10.BR cumulus cells ($P = .03$). The level of *Cyp19a1* mRNA is very low in postovulatory cumuli oophori and almost 2-fold higher in the cells surrounding oocytes of B10.BR(Y^{del}) females than in the cells surrounding gametes of B10.BR females ($P = .004$).

As illustrated in Figure 2, COCs ovulated by B10.BR(Y^{del}) females secrete more progesterone during incubation in vitro than COCs ovulated by females from the control B10.BR strain. The difference is statistically significant ($P = .002$). Because of marginal aromatase activity in postovulatory cumulus cells (Figure 1), the amount of estradiol released to the medium by cultured COCs appeared to be below the detection level.

Despite the selection of exclusively viable spermatozoa and their subsequent incubation under capacitating conditions, in all experiments only a fraction of male gametes presented typical staining of their acrosomal region with P-BSA-FITC complex (Figure 3). This fraction was significantly less numerous for spermatozoa of B10.BR-Y^{del} males than for spermatozoa produced by B10.BR males ($P < .001$): 40.27% of positively stained B10.BR-Y^{del} spermatozoa versus 59.80% of positively stained B10.BR spermatozoa (Figure 4).

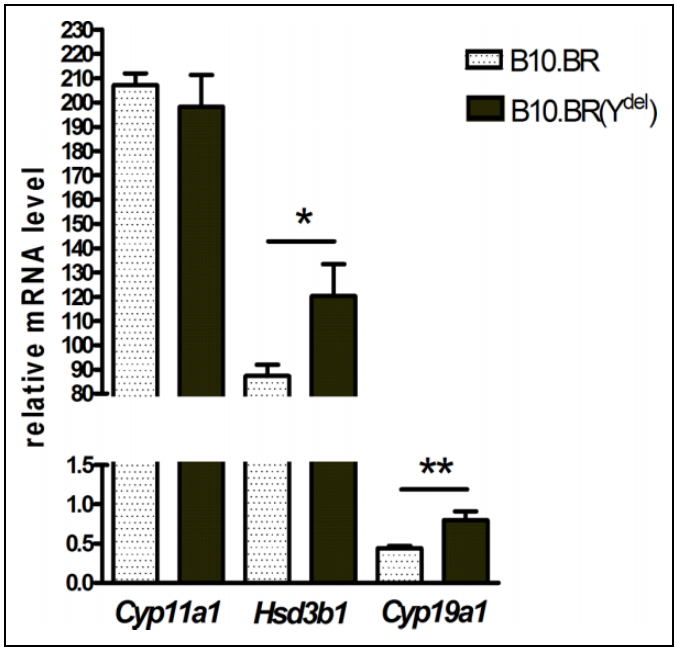

Figure 1. Messenger RNA levels of *Cyp11a1*, *Hsd3b1*, and *Cyp19a1* genes in relation to the endogenous reference gene *Actb* in cumulus cells surrounding ovulated oocytes of B10.BR and B10.BR(Y^{del}) females ($2^{-\Delta Ct}$ values $\times$ 10^3 $\pm$ standard error of the mean [SEM]), $^*P = .03$ and $^{**}P = .004$; n = 6.

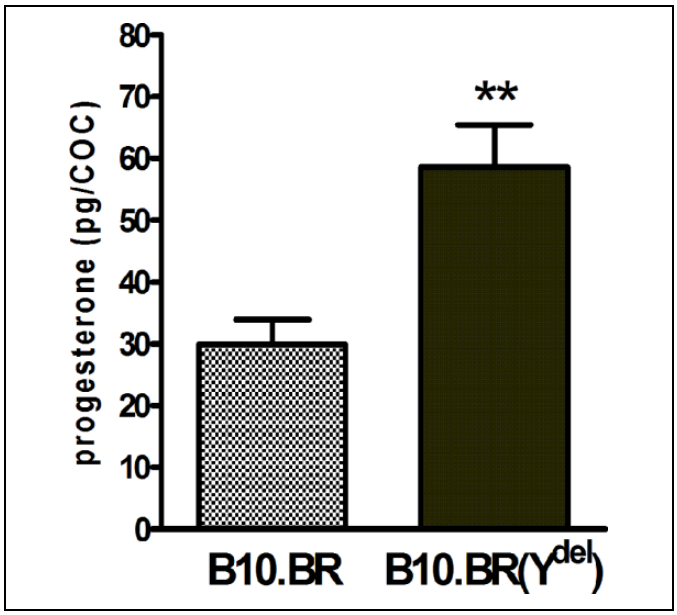

Figure 2. Amount of progesterone (pg) secreted by ovulated B10.BR and B10.BR(Y^{del}) COCs during 4.5 hours incubation in vitro (mean $\pm$ standard error of the mean [SEM]), $^{**}P = .002$; for both groups of females n = 10 independent incubations of minimum 20 COCs. COC indicates cumulus–oocyte complex.

Discussion

Previous experiments carried out in our laboratory have revealed many subtle but noticeable differences in reproductive qualities of females from the congenic B10.BR and B10.BR-

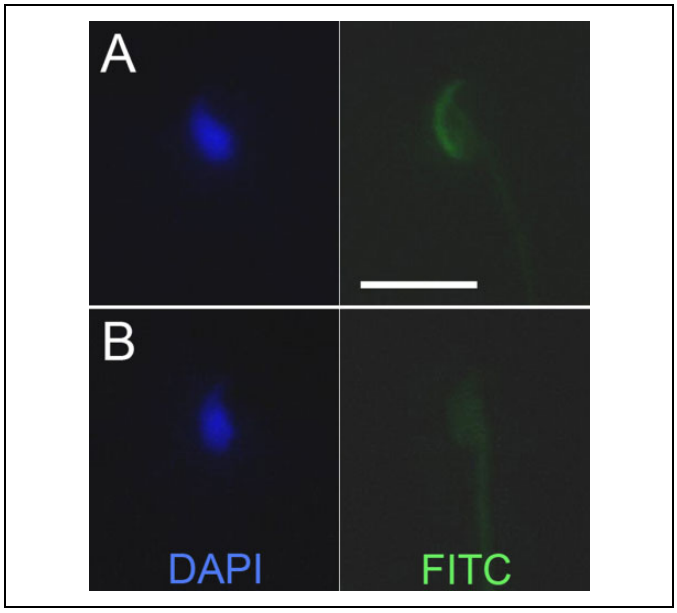

Figure 3. Exemplary spermatozoa positive (A) and negative (B) for staining with progesterone 3-(o-carboxymethyl)oxime: bovine serum albumin-fluorescein isothiocyanate conjugate (P-BSA-FITC) complex. Scale bar = 10 μm.

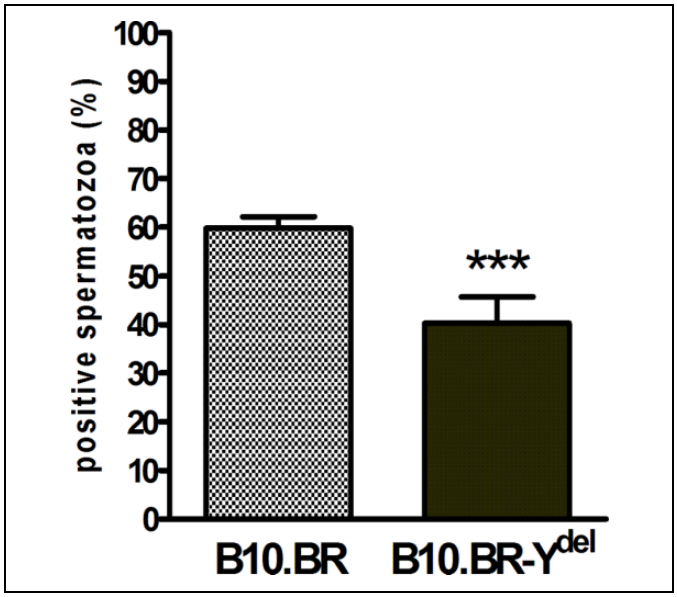

Figure 4. Percent of B10.BR and B10.BR-Y^{del} spermatozoa positive for staining with progesterone 3-(o-carboxymethyl)oxime: bovine serum albumin-fluorescein isothiocyanate conjugate (P-BSA-FITC) complex (mean ± standard error of the mean [SEM]), ***$P < .001$; n = 5.

Y^{del} strains of mice.[14-16] These differences between genetically identical females were explained by indirect influence of paternal Y chromosomes that participate in the establishment of heritable epigenetic marks in X-bearing sperm. Cumulus cells surrounding oocytes of B10.BR(Y^{del}) females just prior and after ovulation present more prostaglandin EP2 receptors and produce more prostaglandin E_2 than analogous cells of B10.BR females.[16] Prostaglandin signaling plays very important role in female reproduction, promoting ovulation[40,41] and keeping ovulated COCs in competence for fertilization.[42] Additionally, prostaglandin E_2, acting via specific membrane receptors on cumulus cells, stimulates their steroidogenic activity.[21,22] The present study shows that ovulated COCs of both B10.BR and B10.BR(Y^{del}) females characterize with typical for their stage expression of genes encoding key steroidogenic enzymes.[43,44] High expression of *Cyp11a1* and *Hsd3b1* genes and very low expression of *Cyp19a1* gene confirm that postovulatory COCs are steroidogenically active and synthesize predominantly progesterone.[25-28] Simultaneously, COCs of B10.BR(Y^{del}) females exhibit higher activity of *Hsd3b1* gene (Figure 1) and release more progesterone during in vitro incubation (Figure 2) in comparison with COCs of control B10.BR females. Above-mentioned regularities imply that, according to our expectations, increased prostaglandin signaling in cumulus cells surrounding ovulated oocytes of B10.BR(Y^{del}) females is associated with increased synthesis of progesterone in these cells. Intensive progesterone production by COCs of B10.BR(Y^{del}) females can influence positively their fertilization efficiency. In oviducts, progesterone secreted by cumulus cells is a well-characterized sperm chemoattractant that not only guide male gametes to oocytes but additionally stimulates their capacitation, hyperactivated motility, rise of intracellular Ca^{2+} concentration, and acrosomal reaction.[29-35,45] Expression level of *Cyp19a1* gene (which is likely to correspond with intensity of testosterone aromatization to 17β-estradiol) is also significantly higher in COCs of B10.BR(Y^{del}) females than in COCs of B10.BR females (Figure 1). This characteristic of COCs of females sired by males with the Y chromosome deletion has presumably smaller physiological relevance. Cumulus cells surrounding ovulated oocytes secrete only slight amounts of estradiol[27,28] (in the present study below the detection level). Besides, effect of estradiol on sperm function is ambiguous, and various studies concerning this problem gave conflicting results.[33,46-50]

Classically, steroids bind to intracellular receptors that induce various transcriptional events. In the case of transcriptionally inactive spermatozoa, they interact with specific cell surface receptors initiating rapid second messenger-mediated responses. Progesterone secreted by cumuli oophori is the predominant steroid that acts on spermatozoa in the female genital tract and considerably facilitates fertilization.[32,33,35] Staining of mouse, stallion, and human spermatozoa with cell-impermeable fluorescein-tagged progesterone-BSA complex (P-FITC-BSA) revealed that progesterone receptors are masked by coating factors in freshly ejaculated sperm and become available after incubation under capacitating conditions, but only in a limited proportion of male gametes.[39,51,52] Similarly, in the present study, we obtained positive staining of progesterone receptors just for a fraction of sperm derived from B10.BR and B10.BR-Y^{del} males. The sperm cells that were negative for P-FITC-BSA staining lack progesterone receptors or alternatively receptors in their membranes remained covered

because of inefficient passing through the capacitating process.[39,52] Availability of progesterone receptors is very important for sperm function. Experiments in vitro with human spermatozoa showed very strong correlation between positive staining of progesterone receptors and ability to undergo acrosome reaction. Gametes of men with various fertility disorders characterize with definitely lower frequency of P-FITC-BSA binding than gametes of fertile men. Therefore, visualization of progesterone receptors at sperm surfaces has been proposed as a simple and reliable method for the evaluation of their functional competence.[51,53] The present study demonstrates that also in the mouse, proportion of spermatozoa positively stained with P-FITC-BSA complex is a good indicator of their general quality. This proportion appeared to be significantly lower for sperm of males with the Y-chromosome deletion than for sperm of control males (Figure 4). Gametes of B10.BR-Y^{del} males are characterized with frequent morphological and ultrastructural irregularities,[6-8] delay in epididymal maturation,[8] decreased effectiveness of movement,[8,10,11] and lower fertilization efficiency in relation to sperm of B10.BR males.[8,9] Now, the reduced availability of surface progesterone receptors has been added to the list.

Extracellular matrix of cumuli oophori surrounding ovulated oocytes of B10.BR(Y^{del}) females is more resistant and abundant with proteins than matrix of cumuli oophori surrounding oocytes of B10.BR females.[15,16] Simultaneously, COCs of B10.BR(Y^{del}) females synthesize and secrete higher amounts of molecules supporting fertilization, such as prostaglandin and progesterone. Because biochemical signaling sent by cumulus cells is recognized only by competent spermatozoa (presenting proper receptors on their surfaces), the described features of COCs of B10.BR(Y^{del}) females can act together as a selective barrier for male gametes. Indeed, sperm of B10.BR males, probably answering molecular signals derived from cumulus cells, fertilize oocytes of B10.BR(Y^{del}) females with the same efficiency as oocytes of B10.BR females. For spermatozoa produced by B10.BR-Y^{del} males, the firm extracellular matrix of B10.BR(Y^{del}) COCs is, however, a serious obstacle that considerably reduces fertilization ratio.[15] We postulate that low availability of progesterone receptors on sperm of males with the Y-chromosome deletion contributes to the described regularity. B10.BR-Y^{del} spermatozoa may be insufficiently stimulated and hence less effective in disintegration of resistant cumulus layer that surround oocytes of B10.BR(Y^{del}) females.

It was shown that deficiency of MSYq-encoded multicopy genes in various mouse models (carrying spontaneous deletions or specific shSLY transgene) causes postmeiotic predominance of multicopy X-linked genes acting antagonistically to MSYq genes during sperm differentiation.[54] This disruption of balance between X- and Y-linked genes leads to functional advantage of X-bearing sperm[13] and consequently to sex ratio skew in favor of females in the offspring.[1,12] The present study implies that this imbalance in the activity of X- and Y-linked genes in mutant male mice can lead additionally to the strengthening of selective barriers for spermatozoa in their female progeny.

Authors' Note

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Declaration of Conflicting Interests

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