Teratospermia in Domestic Cats Compromises Penetration of Zona-Free Hamster Ova and Cat Zonae Pellucidae

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ABSTRACT: The ability of spermatozoa to bind and penetrate zona-free hamster ova and the zonae pellucidae of domestic cat oocytes in vitro was compared between normospermic (>60% structurally normal spermatozoa per ejaculate) and teratospermic (<40% normal spermatozoa per ejaculate) domestic cats. The effects of culture media (Biggers, Whitten, Whittingham [BWW] versus modified Krebs Ringer bicarbonate [mKRB]) and simple dilution (DR), ejaculate centrifugation, and either resuspension (NS) or swim-up processing (SU) on penetration also were examined. High percentages of structurally normal spermatozoa were bound to zona-pellucida-free hamster ova regardless of the morphological forms in the inseminant. Mean percent normal spermatozoa bound to ova in DR, NS, and SU sperm aliquots from teratospermic male cats were not different (P > 0.05) from similarly treated normospermic aliquots. However, the percent penetration of hamster ova by normospermic ejaculates (10.5%) was superior (P < 0.05) to that of teratospermic ejaculates (2.8%). Although swim-up processing improved percent sperm motility, progressive motility, and normal morphology in teratospermic ejaculates (P < 0.05), no difference was observed in ovum penetration among the DR-treated, NS-treated, and SU-treated spermatozoa (P > 0.05). Culture medium had no effect on sperm binding in the hamster assay, but ovum penetration rate by spermatozoa in the normospermic ejaculates was enhanced (P < 0.05) using mKRB (13.5%) when compared with BWW (7.6%) medium. Spermatozoa from teratospermic cats were capable of binding and penetrating cat zonae; however, sperm–zona interaction (defined as percent of oocytes with spermatozoa binding to or penetrating into the zona) was different (P < 0.05) between normospermic (85.5%) and teratospermic (24.2%) cats. The number of bound spermatozoa per ovum was five-fold less in males producing high proportions of pleiomorphic spermatozoa. Zona penetration was also different between normospermic (58.1%) and teratospermic (16.5%) males. Within cat populations, the DR, NS, and SU treatments had no influence on sperm binding or zona penetration. These results indicate that teratospermia in domestic cats has a detrimental impact on gamete interaction and penetration of zona-free hamster ova and cat zona pellucidae. The in vitro effect is not entirely dependent on absolute numbers of structurally normal spermatozoa but appears to be related to an unidentified characteristic of teratospermic ejaculates.

Key words: Sperm morphology, teratospermia, sperm function, zona-free hamster ova, zona pellucida.

Assessing certain sperm functions, including zona binding and penetration. Ovarian antral follicles are one source of zona-intact ova, and homologous immature oocytes are useful for assessing sperm function in humans (Overstreet et al., 1980) and rodents (Biel and Wassarman, 1980).

The domestic cat is a valuable animal model in biomedical research and in comparative studies of rare Felidae species (Wildt et al., 1986). Many nondomestic felid species ejaculate high proportions (range, 36% to 84%) of pleiomorphic spermatozoa (Howard et al., 1984), which is possibly related to low circulating concentrations of testosterone, but not pituitary, gonadotropin secretion (Wildt et al, 1983, 1987a,b, 1988). In general, the domestic cat ejaculates a high proportion (2% to 30%) of structurally abnormal spermatozoa (Sokja et al, 1970; Wildt et al., 1983); however, certain domestic cats consistently ejaculate high proportions (>60%) of pleiomorphic spermatozoa (Howard et al, 1990). Compared with normospermic male cats, teratospermic males produce comparable serum follicle-stimulating hormone and luteinizing hormone concentrations but lower levels of testosterone.

The overall goal of this study was to develop methods to assess sperm function in felids and, specifically, the impact of teratospermia on in vitro sperm-ovum interaction in domestic cats. Two approaches were compared: penetration of heterologous, zona-free hamster ova and homologous, cat zona pellucidae.

Materials and Methods

Animals and Semen Collection

Adult males from two domestic cat populations producing either high (≥60% per ejaculate; normospermic) or low (<40% per ejaculate; teratospermic) proportions of structurally normal spermatozoa were studied. Details on laboratory maintenance of these animals have been described (Howard et al., 1990). In brief, cats were maintained individually in stainless-steel cages and exposed to approximately 12 hours of natural daylight per day; 8 of these hours were supplemented with artificial fluorescent illumination. Animals were provided with dry, commercial cat food (Purina Cat Chow, Ralston Purina Co, St. Louis, MO) and water ad libitum.

A total of 48 ejaculates (24 normospermic and 24 teratospermic) was collected by electroejaculation from six male cats (3 normospermic, 3 teratospermic males; 8 ejaculates/individual). Each male was anesthetized for semen collection with an intramuscular injection of ketamine-hydrochloride (Vetalar®, Parke-Davis, Morris Plains, NJ; 25 mg/kg). After induction of a surgical plane of anesthesia, a Teflon rectal probe (1 cm in diameter) and an alternating current electroejaculator (PT Electronics, Boring, OR) were used to deliver the electrical stimuli (Howard et al., 1990). The electroejaculation regimen, consisting of a total of 80 electrical stimuli divided into three series, was described previously (Howard et al., 1990).

Seminal Analysis and Processing

Fresh semen from each of the three electroejaculation series was combined and evaluated immediately (Howard et al., 1986, 1990). In brief, this involved measuring ejaculate volume and sperm concentration (Howard et al., 1986), and subjectively assessing percent sperm motility and progressive motility at 37°C by phase-contrast microscopy (250x). Sperm progressive motility was based on a scale of 0 to 5, with 0 being no motility and 5 being rapid, steady, forward progression. Sperm morphology was evaluated by fixing ejaculate aliquots (20 μl) in 1% glutaraldehyde followed by phase-contrast microscopic examination (1000x) of 200 spermatozoa/ aliquot (Howard et al., 1990). Each cell was classified as normal or having one of the following anomalies: abnormal head (macrocephalic, microcephalic, bicephalic, or tricephalic), mitochondrial sheath aplasia (including partial or complete aplasia of the mitochondrial sheath), tightly coiled flagellum, bent midpiece with or without a cytoplasmic droplet, bent flagellum with or without a cytoplasmic droplet, or a proximal or distal cytoplasmic droplet.

Ejaculates containing spermatozoa with at least a 70% motility rating were transferred to 1.5-ml conical tubes (Sarstedt Inc, Princeton, NJ) and processed for coincubation with either hamster ova or domestic cat oocytes. Semen was diluted slowly in 200 μl of either Biggers, Whitten, and Whittingham (BWW) medium containing 3 mg/ml bovine serum albumin (BSA; Biggers et al., 1971) or modified Krebs Ringer bicarbonate (mKRB) medium containing 4 mg/ml BSA (Niwa et al., 1985). BWW medium has been used traditionally for the hamster ovum assay (Rogers, 1985). Comparison of the mKRB medium was based on previous use in our laboratory for processing domestic cat ejaculates for in vitro fertilization (Goodrowe et al., 1988).

To assess the influence of centrifugation, seminal plasma removal, and swim-up separation on sperm traits and subsequent ovum penetration, diluted semen was split into three aliquots: diluted raw (DR), non-swim-up (NS), and swim-up (SU). NS and SU aliquots were centrifuged (300g, 8 minutes), supernatant was discarded, and 50 μl of fresh medium was layered gently onto each sperm pellet. The SU spermatozoa were allowed to migrate into fresh medium, whereas NS spermatozoa were resuspended immediately in supernatant by gentle aspiration-pipetting action. Following a 1-hour preincubation period at room temperature (25°C) for spermatozoal capacitation (Goodrowe et al., 1988a), an aliquot of each of the three sperm suspensions was evaluated for sperm motility, progressive motility, morphology, and concentration. To standardize sperm concentration during the sperm-ovum coincubation period, each sample was diluted to a final concentration of 10 × 10⁶ motile spermatozoa/ml.
Zona-Free Hamster Ovum Assay

A total of 36 electroejaculates (18 normospermic and 18 teratospermic; 6 ejaculates/male) was used to study gamete interaction and penetration of zona-pellucida-free hamster ova by domestic cat spermatozoa. In addition to determining if seminal processing influenced heterologous ovum penetration, the effect of culture medium was assessed by comparing BWB and mKRB media.

To obtain ova, immature hamsters were administered an intraperitoneal injection of 40 IU pregnant mares’ serum gonadotropin (PMSG; Sigma Chemical Co, St. Louis, MO) and 25 IU human chorionic gonadotropin (hCG; Sigma Chemical Co) 56 hours later. Fifteen hours after hCG, oviducts were retrograde-flushed with either BWB or mKRB medium. Hamster ova were recovered and treated with 0.1% hyaluronidase (Type I-S, Sigma Chemical Co) and 0.1% trypsin (Sigma Chemical Co) to remove cumulus cells and zona pellucida, respectively (Rogers, 1985). Following preincubation of spermatozoa, zona-free hamster ova (20 ova/drop) were added to a 20-μl drop of diluted spermatozoal suspension (2 × 10⁶ motile sperm cells/drop). After a 3-hour incubation period in a 5% carbon dioxide in air, humidified environment (37°C), hamster ova were washed three times and mounted on a microscopic slide as described previously (Rogers, 1985). Using phase-contrast microscopy (400X), hamster ova were examined and scored for the following: 1) sperm–ovum interaction; 2) numbers of bound spermatozoa per ovum; 3) morphological structure of bound spermatozoa; and 4) ovum penetration. Sperm–ovum interaction was defined as the percentage of ova with spermatozoa either binding to the vitelline membrane or penetrating the vitellus and having at least one swollen sperm head. Spermatozoa remaining on the vitelline membrane following three washes were recorded as bound. Morphological structure of bound spermatozoa was classified as normal or having one of the structural abnormalities described earlier. Ovum penetration was defined by the presence of a swollen or decondensed sperm head with a corresponding flagellum in the vitellus. Penetration results were expressed as: 1) percent penetration rate, which was calculated as the number of ova penetrated divided by the total number of ova inseminated times 100; and 2) penetration index, which was calculated as the total number of decondensed sperm heads divided by the total number of ova inseminated.

Zona-Intact Domestic Cat Oocyte Assay

Sperm function and zona penetration of cat oocytes were assessed in 12 electroejaculates (6 normospermic and 6 teratospermic; 2 ejaculates/male). Semen was diluted in mKRB and processed as described for the hamster assay. Each diluted ejaculate was split into three aliquots (DR, NS, and SU), incubated at 25°C for 1 hour, and diluted to a final concentration of 10 × 10⁶ motile spermatozoa/ml.

Domestic cat ovaries, obtained by ovariectomy, were placed immediately in physiological saline and maintained at 5°C for 1 to 3 hours before processing. Ovaries were punctured repeatedly with a 22-gauge needle to release cumulus-oocyte complexes into the surrounding mKRB medium. Oocytes of various stages of maturity were released. Only oocytes with homogeneously dark vitelli and tightly compacted corona radiata and cumulus cell masses, previously described as immature (Goodrowe et al., 1988b), were selected for insemination. Approximately 10 to 20 viable oocytes at the desired maturation stage were recovered from each ovary. To allow loosening of the cumulus oophorus cells, oocytes were transferred into fresh mKRB and cultured under light-tight paraffin oil (Fisher Scientific Co, Pittsburgh, PA) in a 5% carbon dioxide in air, humidified environment (37°C) for 16 hours. To visualize zona penetration more readily, cumulus cells were removed by transferring oocytes to mKRB containing 0.2% bovine testicular hyaluronidase (Type I-S, Sigma Chemical Co) for 15 minutes (37°C). Oocytes were washed three times in mKRB and manipulated individually using a siliconized micropipette with an opening of similar diameter to the zona pellucida (~162 μm). This was essential for removal of all residual cumulus cells attached to the zona pellucida.

For insemination, 20 cat oocytes were placed in a 35 × 10 mm culture dish containing a 20-μl drop of the processed sperm suspension (2 × 10⁶ motile sperm/drop). After a 3-hour incubation period in a 5% carbon dioxide in air, humidified environment (37°C), ova were washed three times in fresh mKRB medium using a fine bore, siliconized micropipette for removing loosely attached spermatozoa. Ten oocytes were transferred to a concave, single-well microscopic slide containing 40 μl mKRB medium. Each oocyte was examined individually by differential interference-contrast microscopy (320X) using a micromanipulator consisting of a holding pipette for stabilizing and manipulating the oocyte during examination. Spermatozoa remaining on the zona pellucida were recorded as bound, whereas spermatozoa within the zona pellucida were classified as penetrating. Percent zona penetration was determined as the number of ova with spermatozoa penetrating into the zona pellucida divided by the total number of ova inseminated times 100. Zona penetration index was calculated as the total number of spermatozoa penetrating into the zona pellucida divided by the number of ova inseminated. Sperm–zona interaction (defined as the percentage of ova with spermatozoa binding to or penetrating into the zona pellucida) was used as an index of the functional ability of spermatozoa to bind and penetrate the zonae pellucidae of homologous oocytes.
Statistical Analysis

The hamster ovum penetration study was a split-split plot design in which the whole plot was the sperm morphology population (normospermic vs. teratospermic groups), the sub-plot was medium (BWW vs. mKRB), and the sub-sub plot was sperm treatment (DR vs. NS vs. SU treatments).

The design for the cat zona penetration study was similar, with the whole plot being the sperm morphology population (normospermic and teratospermic) and the sub-plot being sperm treatment (DR, NS, and SU treatments). Data were analyzed using a general linear models program (SAS, 1986). Dependent variables presented as percentages (e.g., the percentage of structurally normal spermatozoa) were analyzed using raw and arcsine transformed data. Because analysis of variance revealed no interpretive difference between the raw and transformed data, results were presented as least squares means ± SEM of raw values. When a significant variance value was calculated (P < 0.05), differences among sperm treatment means were determined by the least significant difference (LSD) multiple-comparison procedure. Comparisons within each sperm morphology population included differences among sperm treatments within each medium and among both media. Each SEM was based on pooled variances.

Results

Zona-Free Hamster Ovum Assay

Ejaculate and Inseminant Characteristics Between Populations—Data concerning seminal traits and the influence of seminal processing on sperm motility, progressive motility, concentration, and normal morphology in normospermic and teratospermic cats are presented in a parallel report (Howard et al., 1990). To facilitate interpretation of the sperm–ovum interaction data, relevant information on ejaculate and inseminant characteristics from that article is presented here and in Tables 1 and 3. Seminal traits, including ejaculate volume and the total number of motile spermatozoa per ejaculate, were similar (P > 0.05) between the normospermic (124.1 ± 9.5 μL, 17.0 ± 3.8 × 10⁶, respectively) and teratospermic (97.4 ± 9.5 μL, 24.6 ± 3.8 × 10⁶, respectively) cats. Percent sperm motility and progressive motility also were similar (P > 0.05) between populations; however, teratospermic males consistently produced ejaculates with fewer (P < 0.05) structurally normal spermatozoa than normospermic males. In both normospermic and teratospermic males, the addition of either BWW or mKRB to raw ejaculates increased (P < 0.05) the number of structurally abnormal spermatozoa (Table 1), primarily by increasing the number of cells with a bent flagellum (Howard et al., 1990). Compared with the DR and NS treatments, the swim-up (SU) technique improved (P < 0.05) motility ratings and recovery of structurally normal, motile spermatozoa for both normospermic and teratospermic males (Table 1).

Sperm-Ovum Interaction and Penetration of Hamster Ova by Cat Spermatozoa—Spermatozoa from both cat populations were capable of interacting similarly (P > 0.05) with zona-free hamster ova as demonstrated by high sperm–ovum interaction values in normospermic (93.8 ± 0.1%) and teratospermic (96.2 ± 0.1%) cats. Neither culture medium nor the DR, NS, and SU treatments influenced (P > 0.05) sperm–ovum interaction ratings in either cat population.

Spermatozoa from normospermic and teratospermic cats penetrated hamster ova as demonstrated by the presence of a decondensed sperm head within the cytoplasm (Fig 1). The penetration index and penetration rate for normospermic males were superior (P < 0.05) to that of teratospermic males (Table 2). These findings were consistent within medium and sperm processing treatment groups. Although

| TABLE 1. Influence of sperm treatment on sperm motility and structural morphology* |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Sperm treatment                | Undiluted raw   | Diluted raw     | Non-swim-up     | Swim-up         |
| Sperm motility (%)†             | 78.7 ± 1.4§     | 70.9 ± 1.4§     | 69.4 ± 1.4§     | 91.2 ± 1.4§     |
| Sperm progressive motility†,‡   | 4.0 ± 0.1§      | 3.6 ± 0.1§      | 3.7 ± 0.1§      | 4.3 ± 0.1§      |
| Normal spermatozoa (%)          |                |                |                |                |
| Normospermic ejaculates         | 74.2 ± 2.1§     | 44.6 ± 2.1§     | 43.5 ± 2.1§     | 69.1 ± 2.1§     |
| Teratospermic ejaculates        | 34.0 ± 2.1§     | 25.4 ± 2.1§     | 27.7 ± 2.1§     | 59.4 ± 2.1§     |

* Results from normospermic and teratospermic domestic cat ejaculates used for co-incubation with zona-free hamster ova and zona-intact domestic cat oocytes. Values are means ± pooled SEM of combined oocyte assays. No difference in sperm traits was detected between the zona-free hamster ovum assay and the zona-intact cat oocyte assay.
† Values are means ± pooled SEM of normospermic (n = 24) and teratospermic (n = 24) ejaculates. No difference in sperm percent motility and progressive motility was detected between the cat populations.
‡ Sperm progressive motility was based on a scale of 0 to 5. 5 = most rapid forward progression.
§, ¶ Within rows, means with different superscripts differ (P < 0.05).
FIG. 1. Penetration of a zona-free hamster ovum by a domestic cat spermatozoon. One swollen sperm head (arrow) with attached flagellum is visible in the vitellus of the ovum. Scale bar = 50 μm.

Ovum penetration rates were similar between the BWW and mKRB treatments in the teratospermic group, the latter medium enhanced (P < 0.05) the ovum penetration rate in the normospermic population (Table 2). The DR, NS, and SU treatments had no effect on penetration rate or index, even though the latter process improved (P < 0.05) sperm motility, progressive motility, and normal cellular morphology of the inseminants.

**Morphological Characteristics of Spermatozoa Bound to Hamster Ova**—A high proportion of spermatozoa bound to the vitelline membranes of hamster ova was structurally normal in the teratospermic (73.3%) and normospermic (78.4%) aliquots. In teratospermic males, the percentage of normal spermatozoa bound to ovum was greater (P < 0.05) than the proportion of normal spermatozoa in the inseminants, regardless of sperm treatment (Table 3). Similar contrasts were observed in the DR and NS aliquots from normospermic ejaculates (data not shown). The proportion

**TABLE 2. Influence of sperm morphology and culture media on zona-free hamster ovum penetration**

<table>
<thead>
<tr>
<th>Ova (total number)</th>
<th>Bound sperm/ovum* (number)</th>
<th>Penetration index†</th>
<th>Penetration rate‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normospermic ejaculates (n = 18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BWW medium</td>
<td>540</td>
<td>29.9 ± 5.7</td>
<td>0.06§</td>
</tr>
<tr>
<td>mKRB medium</td>
<td>525</td>
<td>24.2 ± 5.8</td>
<td>0.15†</td>
</tr>
<tr>
<td>Teratospermic ejaculates (n = 18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BWW medium</td>
<td>536</td>
<td>22.2 ± 5.7</td>
<td>0.04‖</td>
</tr>
<tr>
<td>mKRB medium</td>
<td>540</td>
<td>31.0 ± 5.7</td>
<td>0.02‖</td>
</tr>
</tbody>
</table>

* Values are means ± pooled SEM.
† Penetration index = the total number of decondensed sperm heads divided by the number of ova inseminated.
‡ Penetration rate = the number of ova with decondensed sperm heads divided by the total number of ova inseminated multiplied by 100.
§, †. ‖ Within columns, values with different superscripts differ (P < 0.05).
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TABLE 3. Morphological characteristics of inseminated spermatozoa compared to spermatozoa bound to zona-free hamster ova in teratospermic domestic cats*

<table>
<thead>
<tr>
<th>Teratospermic ejaculates</th>
<th>Inseminated spermatozoa</th>
<th>Bound spermatozoa†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diluted raw</td>
<td>Non-swim-up</td>
</tr>
<tr>
<td>Normal spermatozoa (%)</td>
<td>22.7 ± 2.3‡</td>
<td>25.8 ± 2.3‡</td>
</tr>
<tr>
<td>Abnormal spermatozoa (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal sperm head</td>
<td>2.1 ± 0.1</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Mitochondrial sheath aplasia</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Collar flagellum</td>
<td>10.0 ± 2.0</td>
<td>8.7 ± 1.8</td>
</tr>
<tr>
<td>Bent midpiece with droplet</td>
<td>14.6 ± 1.2</td>
<td>14.3 ± 1.6</td>
</tr>
<tr>
<td>Bent midpiece, no droplet</td>
<td>4.8 ± 0.7</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td>Bent flagellum with droplet</td>
<td>19.9 ± 3.2</td>
<td>18.9 ± 3.4</td>
</tr>
<tr>
<td>Bent flagellum, no droplet</td>
<td>21.7 ± 2.3</td>
<td>21.5 ± 2.3</td>
</tr>
<tr>
<td>Proximal droplet</td>
<td>2.3 ± 0.5</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>Distal droplet</td>
<td>1.8 ± 0.4</td>
<td>1.7 ± 0.3</td>
</tr>
</tbody>
</table>

* Values are means ± SEM of combined media groups (BWW and mKR8).
† Values are means ± SEM of combined sperm treatment groups. The proportion of normal sperm bound to ova was similar among the dilute raw, non-swim-up, and swim-up treatments.
‡ § Within-row means with different superscripts differ (P < 0.05).

of normal spermatozoa bound in the DR, NS, and SU aliquots from normospermic ejaculates incubated in BWW and mKR8 were comparable (P > 0.05) to similarly processed aliquots in the teratospermic group. The specific morphologic types of spermatozoa bound to hamster ova are depicted in Table 3. With one exception, there were at least a few spermatozoa exhibiting each type of abnormality found attached to ova. Only those cells with partial or complete mitochondrial sheath aplasia appeared incapable of binding (Table 3).

Zone-Intact Domestic Cat Oocyte Assay

Ejaculate and Inseminant Characteristics Between Populations—Raw ejaculate traits for each cat population were similar to the values reported for these same males in the characterization study of Howard et al (1990) and used for heterologous fertilization of hamster ova. The number of motile spermatozoa per ejaculate was similar (P > 0.05) between groups, and the mean number of normal cells per ejaculate was greater (P < 0.05) in normospermic than teratospermic males (Table 1). Mean percent sperm motility and progressive motility were similar (P > 0.05) between normospermic and teratospermic cats. As observed in the hamster ovum study, diluting raw ejaculates in mKR8 medium reduced (P < 0.05) the number of structurally normal spermatozoa. The SU treatment, however, improved the proportion of structurally normal spermatozoa within each cat population compared with the DR and NS aliquots and enhanced percent sperm motility and progressive motility (Table 1).

Oocyte Penetration—Spermatozoa from normospermic and teratospermic cats were capable of binding to and penetrating the zona pellucida of homologous oocytes (Fig 2); however, overall sperm–zona interaction was more than two-fold greater for the normospermic group (Table 4). There was at least a three-fold increase (P < 0.05) in the number of bound spermatozoa per ovum and zona penetration index, and more than a three-fold increase (P < 0.05) in zona penetration rate for normospermic compared to teratospermic males (Table 4). The various sperm treatments (DR, NS, and SU) had no influence on any of the interactions, or binding or penetration characteristics examined (Table 4).

Discussion

This study provides the first evidence that teratospermia in domestic cats has a detrimental impact on gamete interaction in vitro. Compared with those of normospermic males, the spermatozoa from teratospermic cats were less capable of penetrating heterologous ova and binding and penetrating homologous zonae pellucidae. The most striking observation was that this compromised ability appeared inherent to the teratospermic ejaculate and was not dependent on the absolute number of structurally defective spermatozoa. Teratospermic ejaculates subjected to swim-up processing (to increase the number of normal sperm forms) were still three to six times less likely to penetrate zona-free hamster ova and almost four times less likely to penetrate zona-intact cat oocytes than similarly treated spermatozoa from normospermic cats. The differences between cat populations were similar to those observed in humans, in which the number of hamster ovum penetrations correlate positively to the number of structurally normal spermatozoa, more so than to other seminal characteristics (Rogers et al, 1983; Marsh et al, 1987; Kruger et al, 1988). In oligospermic men (Aitken
et al., 1982) and in cases of unexplained infertility (Aitken et al., 1983), spermatozoal morphology and fertilizing capacity appear to be closely related. Kot and Handel (1987) reported that structurally abnormal spermatozoa from certain strains of mice bind to the zonae pellucidae of conspecific ova at a much lower frequency than might be predicted. Studies also have related decreased human ovum penetration rates to an increased incidence of abnormal spermatozoa in the insenminant (Mahadevan and Trounson, 1984). Studies in our laboratory have demonstrated that many factors influence the ability of cat follicular oocytes to fertilize in vitro and develop into biologically competent embryos in vivo (Goodrowe et al., 1988b; Miller et al., 1988; Johnston et al., 1989, 1991). It now is apparent that abnormally high numbers of pleiomorphic spermatozoa also would decrease IVF success rate in this species.

In the context of sperm penetration, both the zona-free hamster ovum and cat zonae pellucidae assays produced similar results. The concordance of the bioassays in demonstrating functional differences between spermatozoa from normospermic and teratospermic ejaculates confirmed the results and also indicated that these approaches were complementary. Numerous studies have demonstrated that the zona-free hamster ovum assay correlates positively with the penetration of zona-intact human oocytes (Overstreet et al., 1980; Wolf et al., 1983; Margalioth et al., 1986; Kruger et al., 1988). In the current study, however, the hamster assay was ineffective in distinguishing the binding dysfunction in the teratospermic cats, which was readily apparent when homologous, zona-intact oocytes were used. There appears to be a fundamental functional defect in spermatozoa from teratospermic cats, which is expressed as a diminished ability of the spermatozoa to interact with, bind to, and penetrate the zona pellucida.

The various sperm treatments, including swim-up processing, had no effect on sperm–ovum binding or penetration in either bioassay. The swim up method, however, is known to enhance cat sperm motility and morphology in vitro (Howard et al., 1990). Reducing the opportunity for abnormal spermatozoa to participate in fertilization may reduce the risk of producing defective or chromosomally abnormal embryos. The penetration rate and index were increased in the normospermic ejaculates exposed to hamster ova in the presence of mKRB medium. Fertilization in vitro is facilitated by albumin (Miyamoto and Chang, 1973; Hoppe and Whitten, 1974), and this protein component dif-
TABLE 4. Penetration of zona-pellucida-intact, domestic cat oocytes by normospermic and teratospermic domestic cat spermatozoa

<table>
<thead>
<tr>
<th></th>
<th>Normospermic ejaculates (n = 6)</th>
<th>Teratospermic ejaculates (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of ova</td>
<td>360</td>
<td>360</td>
</tr>
<tr>
<td>inseminated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm–zona interaction</td>
<td>65.3 ± 0.1§</td>
<td>24.2 ± 0.1§</td>
</tr>
<tr>
<td>(%)§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of bound</td>
<td>1.0 ± 0.1§</td>
<td>0.2 ± 0.1§</td>
</tr>
<tr>
<td>sperm/ovum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zona penetration index†</td>
<td>1.7§</td>
<td>0.3†</td>
</tr>
<tr>
<td>Zona penetration rate‡</td>
<td>58.1%§</td>
<td>16.9%§</td>
</tr>
</tbody>
</table>

* Values are means ± SEM. Sperm–zona interaction was the percentage of ova with spermatozoa binding to or penetrating into the zona pellucida.
† Zona penetration index = the total number of spermatozoa penetrating into the zona pellucida divided by the number of ova inseminated.
‡ Zona penetration rate = the number of ova with spermatozoa penetrating into the zona pellucida divided by the total number of ova inseminated times 100.
§, † Within rows, values with different superscripts differ (P < 0.05).

...spermatozoa observed in certain populations of androgen-deﬁcient domestic or nondomestic ﬁelds may be the result of defective spermatogenesis or epididymal dysfunction (Howard et al., 1990). In all mammals studied at this time, critical androgen-dependent changes occur during epididymal maturation and sperm storage (Orgebin-Crist and Fournier-Deipsch, 1982). Because this critical interval is accompanied by signiﬁcant changes in nuclear chromatin and DNA stabilization (Huret, 1986), defective epididymal function may be a cause of the diminished ability of spermatozoa to interact with ova and undergo nuclear decondensation. In the human, an apparent correlation exists between the progressive accumulation of epididymal proteins on the sperm surface during transit and the simultaneous ability to penetrate zona-free hamster oocytes (Moore et al., 1983). Deposition of these proteins on the sperm surface also has been correlated with the acquisition of zona pellucida binding capacity and fertilizing ability (Orgebin-Crist and Fournier-Deipsch, 1982; Cuasnicu et al., 1984). These studies suggest that the compromised binding and penetrating ability of spermatozoa from the androgen-deﬁcient, teratospermic cats may be related to epididymal dysfunction and a deﬁciency or alteration in sperm surface proteins.

In summary, this investigation demonstrated that cat spermatozoa from teratospermic ejaculates were compromised in three initial stages of sperm–ovum interaction: binding to the zona, penetration into the zona pellucida, and fusion with the oolemma. It is encouraging, however, that spermatozoa recovered from cats producing such high percentages of plieiomorphisms were still capable of gamete interaction, albeit at a compromised rate. Assuming that such a finding is not species speciﬁc, this observation is relevant to conservation biology since certain species, such as the cheetah (Wildt et al., 1987a), leopard (Wildt et al., 1988), and Asiatic lion (Wildt et al., 1987b), consistently produce a high proportion (>70%) of abnormal spermatozoa per ejaculate. Our studies of the Florida panther (Felis concolor coryi), a highly endangered puma subspecies, reveal that males routinely produce 94% sperm pleiomor-
phisms per ejaculate, with an average of 47% structurally defective acrosomes. Spermatozoa from individual males producing as many as 99% total pleiomorphisms and 63% defective acrosomes are still capable of fertilizing homologous oocytes in vitro (Miller et al., 1990). The zona-free hamster oocyte and zona-intact cat oocyte bioassays described here will be important tools for assessing numerous factors controlling fertilization and will improve our overall understanding of the impact and, perhaps, etiology of terato- spermatism.

Acknowledgments

The authors thank Dr. Mark A. Barone, Karen J. Clingerman, and Sandy L. Hurbut for their valuable expertise and technical assistance. We also are grateful to the following veterinary clinics for their cooperation and for providing cat ovaries: Maple Springs Veterinary Office, Gaithersburg, MD; Montgomery Animal Hospital, Rockville, MD; Peachtree Veterinary Clinic, Beallsville, MD; and Seneca Valley Veterinary Clinic, Gaithersburg, MD.

References

Aitken RJ, Best FSM, Richardson DW, Djahanbakhch D, Mortimer D, Templeton AA, Lees MM. An analysis of sperm function in cases of unexplained infertility: conventional criteria, movement characteristics and fertilizing capacity. Fertil Steril. 1982;38:212–221.


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