Male accessory sex gland secretions affect oocyte Ca\(^{2+}\) oscillations during in-vitro fertilization in golden hamsters

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To evaluate the effect of male accessory sex gland secretions on Ca\(^{2+}\) oscillations of oocytes, epididymal or ejaculated spermatozoa recovered from uteri were used to inseminate oocytes. Ca\(^{2+}\) oscillations were measured by Fura 2 fluorescence imaging (F340/F380). We showed that although Ca\(^{2+}\) oscillations induced by ejaculated spermatozoa had a pattern similar to those induced by epididymal spermatozoa, the amplitude of the first Ca\(^{2+}\) transient in the former group was significantly higher (\(P < 0.05\)) and the duration was significantly longer (\(P < 0.01\)). Oocytes inseminated with ejaculated spermatozoa recovered from uteri from males had ampullary glands or ventral prostates removed showed significantly lower Ca\(^{2+}\) oscillations compared to the controls (\(P < 0.05, P < 0.01\) respectively). Moreover, the relative area of the first Ca\(^{2+}\) transient in treatment groups was significantly smaller than the control. In addition, a significantly higher percentage of oocytes (52%) inseminated by spermatozoa from males with all accessory sex glands removed showed non-oscillatory Ca\(^{2+}\) transients, compared to the controls (5%, \(P < 0.05\)). These results indicate that accessory sex gland secretions can affect Ca\(^{2+}\) oscillations. The differences between Ca\(^{2+}\) oscillations induced by epididymal and uterine spermatozoa from males with all accessory sex glands removed suggest that uterine factors may also influence this process.

Key words: Ca\(^{2+}\) oscillations/golden hamster/in-vitro fertilization/male accessory sex glands/oocyte

Introduction

Male accessory sex gland secretions make up most of the volume and chemical components such as zinc, fructose and acid phosphatase and some proteins, of seminal plasma. Although several of these chemical components can be used to indicate the function of the male accessory sex glands, very little is known about the role of these components in promoting the fertilizing capacity of spermatozoa (Setchell et al., 1994). In mammals, a spermatozoon acquires fertilizing ability in the epididymis, but the degree to which male accessory sex gland secretions affect male fertility is controversial (Henault et al., 1995). In vivo it is very difficult to show whether male accessory sex gland secretions affect fertility in humans. In the rodent model, removal of some or all of the male accessory sex glands reduces fertility in the mouse (Pang et al., 1979; Peitz et al., 1986), rat (Queen et al., 1981) and hamster (Chow et al., 1986), even though fertilization rate is not affected (Chow et al., 1994; Ying et al., 1998).

In-vitro studies show that the fertilization rate of epididymal spermatozoa from rats (Shalgi et al., 1981), cats (Niwa et al., 1985) and pigs (Nagai et al., 1984) is higher than that of ejaculated spermatozoa. Antifertility factors from seminal plasma have been described for several species including human (Baas et al., 1983; Iwamonto and Gagnon, 1988; Iwamonto et al., 1992). These factors are believed to inhibit sperm motility, capacitation and acrosome reaction, and eventually to interfere with fertilization (Van Der Ven et al., 1982; Audhya et al., 1987; Robert and Gagnon, 1996). On the other hand, there are also reports which show that some proteins which bind with sperm plasma membrane during ejaculation are beneficial to fertility (Miller et al., 1990; Killian et al., 1993). Recent studies show that prostate secretions in some species including rabbit, rat and human contain a fertilization-promoting peptide (FPP) (Cockle et al., 1989; Green et al., 1996). FPP can significantly promote the capacitation of epididymal mouse spermatozoa and human ejaculated spermatozoa and increase the percentage of fertilized oocytes in vitro but not acrosome reaction. Thus it is believed that FPP could stimulate male fertility (Green et al., 1994, 1996).

In many studies, effects of male accessory sex gland secretions on male fertility are mainly focused on the function of spermatozoa, or the ability of spermatozoa to fertilize oocytes and the ability of the zygotes to continue to develop into offspring in vivo or blastocysts in vitro. Relatively little is known about the effect of ASG secretions on the subtle events during oocyte activation. Sometimes spermatozoa may penetrate oocytes and form normal-appearing zygotes but fail to initiate subsequent embryonic development properly (Chow et al., 1994; Ying et al., 1998). This has been illustrated by in-vitro and in-vivo studies in which male accessory sex glands have been shown to affect DNA replication of zygotes in the first cell cycle (Eid et al., 1994; Ying et al., 1998).

It is well known that gamete fusion initiates a cascade of events in the oocyte, termed oocyte activation, which blocks polyspermic fertilization and launches the fertilized oocyte...
onto a path leading to DNA synthesis and further embryonic development (Whitaker and Patel, 1990; Kline and Kline, 1992). It is also generally accepted that the fusion of the oocyte with the fertilizing spermatozoa is the physiological trigger of oocyte activation. However, the mechanism by which spermatozoa activate the oocyte is still a matter of controversy. Ca\(^{2+}\) is the major intracellular signalling molecule in somatic cells. Intracellular Ca\(^{2+}\) oscillations play a central role in oocyte activation (Fissore et al., 1992; Xu et al., 1994). Some studies have indicated that a non-oscillatory Ca\(^{2+}\) increase, such as during parthenogenetic activation, often leads to an early embryonic developmental arrest (Sun et al., 1992), whereas repetitive Ca\(^{2+}\) oscillations result in a viable embryo (Kline and Kline, 1992).

Using the golden hamster as a model, we have demonstrated that the ampullary gland and ventral prostate may play an important role in the regulation of male fertility (O et al., 1988; Chow et al., 1994). Male hamsters with either ampullary glands or ventral prostates removed had reduced fertility, which was found to be related to retarded embryonic development and lower implantation rate (O et al., 1988; Chow et al., 1994). Recently we reported that the removal of male ampullary glands or ventral prostates delayed zygotic DNA replication in the first cell cycle during in-vivo fertilization (IVF) (Ying et al., 1998). We postulate that the modification of the sperm plasma membrane by male accessory sex gland secretions might affect the initiation of embryonic development by interfering with signal transduction at the time of oocyte activation. The objective of this paper is to study the patterns of intracellular Ca\(^{2+}\) oscillations in oocytes activated by spermatozoa ejaculated from males whose accessory sex glands had been totally or partially removed.

**Materials and methods**

**Animals**

Randomly bred golden hamsters (*Mesocricetus auratus*) were fed with standard food and tapwater *ad libitum*, and maintained in the Laboratory Animal Unit, Faculty of Medicine, The University of Hong Kong, under a 14 h light/10 h dark regime (light 11:00–01:00). Female hamsters, aged 10 weeks, were checked daily for vaginal secretion for at least two consecutive normal oestrous cycles before mating.

**Reagents**

Unless otherwise stated, all inorganic and organic chemicals were purchased from Sigma Chemical Company (St Louis, MO, USA).

**Media**

Tyrode’s albumin–lactate pyruvate medium (TALP) was used for collection and manipulation of oocytes and for IVF. The composition of TALP medium was: 114.0 mM NaCl, 3.2 mM KCl, 2.0 mM CaCl\(_2\), 0.5 mM MgCl\(_2\), 25.0 mM Na\(_2\)HCO\(_3\), 0.4 mM NaH\(_2\)PO\(_4\), 5.0 mM glucose, 10.0 mM sodium lactate, 0.1 mM sodium pyruvate, 0.35 mg/ml gentamicin sulphate and 3 mg/ml bovine serum albumin BSA. For capacitation of hamster spermatozoa, modified TALP (m-TALP: TALP supplemented with 0.5 mM taurine, 0.05 mM adrenaline and 15 mg/ml BSA) was used.

**Surgery**

Male accessory sex glands were removed from 7–8 week old male hamsters following established methods (Chow et al., 1986) to give the following four groups: SH, sham-operated controls; AGX, bilateral excision of the ampullary glands; VPX, bilateral excision of the ventral prostates; TX, bilateral excision of the ampullary glands, ventral prostates, dorsolateral prostates, coagulating glands and seminal vesicles. The operated males were used for mating 4 weeks after surgery and the success of the operation was checked at the end of the experiments. Each female hamster was mated with one surgically treated male on the day of oestrus for 15 min. Seven or eight operated males were used for each experimental group.

**Collection of oocytes**

Naturally ovulating female hamsters were killed by chloroform inhalation on the day of oestrus. About 10–12 oocytes were flushed from oviducts of one female with 0.05 mg/ml hyaluronidase in BSA-free TALP pre-equilibrated with 5% CO\(_2\) at 37°C and then treated with 0.05 mg/ml trypsin in BSA-free TALP to remove zona pellucida. The zona-free oocytes were loaded with 3.3 µM Fura 2-AM (diluted from a 330 µM Fura 2 stock solution in dimethyl sulphoxide) in TALP which contained the detergent pluronic F-127 (0.04%) for 30 min before insemination. These Fura 2-loaded oocytes were immediately used for the detection of Ca\(^{2+}\) oscillations.

For IVF, adult female golden hamsters were superovulated with 40 IU pregnant mare’s serum gonadotrophin on the day of oestrus followed by 30 IU human chorionic gonadotrophin (HCG) 55 h later. Oocytes were collected 16 h after HCG.

**Preparation of spermatozoa**

**Collection of epididymal spermatozoa**

The epididymis was removed from 10–14 week old male hamsters and cleared of blood and connective tissues. A small cut was made on the tubules of the cauda epididymis to release spermatozoa. About 20–30 µl of this dense sperm mass was transferred to the bottom of a test tube (12 mm×55 mm) and 2 ml of m-TALP medium was gently added. The test tube was allowed to stand for 5 min at 37°C. The upper 1 ml of sperm suspension was transferred to a culture dish (10 mm×35 mm) and the concentration of spermatozoa was adjusted to 3×10\(^5\)/ml. Spermatozoa were incubated under mineral oil in 5% CO\(_2\) and 95% O\(_2\) at 37°C for 4–5 h for capacitation.

**Collection of ejaculated spermatozoa from uteri**

About 20–30 µl of sperm mass was collected from uterine horns of 8–12 week old female hamsters 30 min after mating with operated males. Spermatozoa were processed and incubated for capacitation as in previous section.

**In-vitro fertilization**

Cumulus–oocyte complexes were collected from oviducts of superovulated females and transferred to 1 ml of TALP medium. Oocytes were co-incubated with capacitated spermatozoa (3.0×10\(^5\) spermatozoa/ml) under mineral oil for 4–5 h. They were then transferred to new medium and cultured for another 15 h. The oocytes were examined for fertilization with a phase contrast microscope. An oocyte was recorded as being fertilized when both male and female pronuclei had formed.

**Insemination and measurement of Ca\(^{2+}\) oscillations**

Ca\(^{2+}\) oscillations of oocytes were measured according to previously reported methods (Ben-Yosef et al., 1993; Maleszewski et al., 1995) with modifications. Briefly the Fura 2-loaded oocytes were transferred to 100 µl of BSA-free TALP buffered with HEPES covered with...
mineral oil on a glass coverslip (~20 mm diameter) which had been pretreated with polylysine (0.5 mg/ml) to make oocytes adherent. The glass coverslip was fitted into a stainless steel-welled chamber, mounted on a Zeiss inverted epifluorescence microscope and heated to 37°C by a thermostatically controlled hot plate. To prevent polyspermic penetration, only a small amount of sperm suspension (2–3 µl) was introduced to the surface of the oocyte.

A Metafluor Imaging System (Universal Corporation, USA) was used for fluorescence recordings. Fluorescence signal was displayed as the ratio of fluorescence intensity for 340:380 nm excitation wavelengths after background subtraction. Emitted fluorescence was recorded at each excitation wavelength and the ratio calculated once for each excitation wavelength after background subtraction. Emitted fluorescence was used for fluorescence recordings. Fluorescence signal was displayed as the ratio of fluorescence intensity for 340:380 nm excitation wavelengths after background subtraction. Emitted fluorescence was recorded at each excitation wavelength and the ratio calculated once for each excitation wavelength after background subtraction.

The addition of spermatozoa to zona-free naturally ovulated oocytes resulted in a microscopically visible sperm attachment. Immediately following sperm attachment, oocytes were continuously monitored. Ca2+ oscillations of oocytes induced by epididymal spermatozoa in golden hamster have been described elsewhere (Swann, 1990; Miyazaki et al., 1992; Maleszewski et al., 1997). In the present study, epididymal spermatozoa induced Ca2+ oscillations in 65.4% (17 of 26) of the oocytes (Table II). The first Ca2+ transient varied from almost immediately (8 s) to as long as 10 min following the addition of spermatozoa. The first Ca2+ transient varied in both intensity and duration (0.95 ± 0.41 and 40.9 ± 10.0 s, respectively, n = 17, Table II). This elevation was followed by subsequent regular Ca2+ transients at intervals of 1–3 min. Characteristically, the first Ca2+ transient in each oocyte was of larger amplitude than later ones (Figure 1A). Ca2+ oscillations usually subsided in 30–50 min, during which the baseline gradually drifted upward due to Fura 2 dye bleaching.

Spermatozoa (recovered from uterus) from sham-operated males induced Ca2+ oscillations in 86.4% (19 of 22) of the oocytes (Table II). This was slightly higher, but not statistically significant compared with that induced by epididymal spermatozoa. Ca2+ oscillations induced by uterine spermatozoa (Figure 1B) had a pattern similar to those induced by epididymal spermatozoa (Figure 1A). However, the amplitude of the first Ca2+ transient was significantly higher (P < 0.05), while the duration was longer (P < 0.01) compared with those induced by epididymal spermatozoa (Table II).

The following sets of data were collected from each treatment group and analysed: the percentage of IVF; the percentage of eggs showing Ca2+ oscillations; the number of sperm fused in each oocyte; the first peak ratio amplitude in Ca2+ transients; the first peak duration in Ca2+ transients; the first peak area of Ca2+ transients and duration of peak intervals between Ca2+ transients. ANOVA or χ²-test was used for statistical analysis, P < 0.05 was considered as significant.

### Table I. Effects of male accessory sex gland secretions on in-vitro fertilization

| Groups | Total no. of eggs | Total no. of fertilized eggs | % of fertilization
<table>
<thead>
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<tbody>
<tr>
<td>SH</td>
<td>839</td>
<td>533</td>
<td>63.8 ± 1.8</td>
</tr>
<tr>
<td>AGX</td>
<td>861</td>
<td>609</td>
<td>65.1 ± 6.6</td>
</tr>
<tr>
<td>VPX</td>
<td>967</td>
<td>614</td>
<td>61.6 ± 7.9</td>
</tr>
<tr>
<td>TX</td>
<td>897</td>
<td>529</td>
<td>60.2 ± 5.6</td>
</tr>
</tbody>
</table>

*Data represent mean ± SEM summarized from five experiments.
SH = sham-operated males; AGX = excision of ampullary glands; VPX = excision of ventral prostates; TX = excision of all major male accessory sex glands.

### Results

#### In-vitro fertilization and polyspermy

In order to find out if the secretions of male accessory sex glands affected IVF, cumulus-intact superovulated eggs were inseminated with ejaculated spermatozoa collected from female uteri 30 min after mating with various operated males. About 60% of eggs were found fertilized (Table I). No significant difference was found between SH control and other treatment groups in terms of fertilization rate.

Polyspermic fertilization, which will affect Ca2+ oscillations of oocytes, is a common phenomenon in zona-free hamster oocytes. To prevent polyspermic fertilization, a very small amount of sperm suspension was introduced to inseminate oocytes. No significant difference was found in terms of the mean number of sperm fusions in each oocyte amongst the experimental groups (Table II).

### Ca2+ oscillations induced by epididymal or ejaculated spermatozoa

The addition of spermatozoa to zona-free naturally ovulated oocytes resulted in a microscopically visible sperm attachment. Immediately following sperm attachment, oocytes were continuously monitored. Ca2+ oscillations of oocytes induced by epididymal spermatozoa in golden hamster have been described elsewhere (Swann, 1990; Miyazaki et al., 1992; Maleszewski et al., 1997). In the present study, epididymal spermatozoa induced Ca2+ oscillations in 65.4% (17 of 26) of the oocytes (Table II). The first Ca2+ transient varied from almost immediately (8 s) to as long as 10 min following the addition of spermatozoa. The first Ca2+ transient varied in both intensity and duration (0.95 ± 0.41 and 40.9 ± 10.0 s, respectively, n = 17, Table II). This elevation was followed by subsequent regular Ca2+ transients at intervals of 1–3 min. Characteristically, the first Ca2+ transient in each oocyte was of larger amplitude than later ones (Figure 1A). Ca2+ oscillations usually subsided in 30–50 min, during which the baseline gradually drifted upward due to Fura 2 dye bleaching.

Spermatozoa (recovered from uterus) from sham-operated males induced Ca2+ oscillations in 86.4% (19 of 22) of the oocytes (Table II). This was slightly higher, but not statistically significant compared with that induced by epididymal spermatozoa. Ca2+ oscillations induced by uterine spermatozoa (Figure 1B) had a pattern similar to those induced by epididymal spermatozoa (Figure 1A). However, the amplitude of the first Ca2+ transient was significantly higher (P < 0.05), while the duration was longer (P < 0.01) compared with those induced by epididymal spermatozoa (Table II).

#### Effects of male accessory sex gland secretions on Ca2+ oscillations

Table II summarizes changes of Ca2+ oscillations of oocytes induced by uterine spermatozoa from males in the different treatment groups. In the four different experiments, a total of 99 oocytes were examined. About 83% of them showed Ca2+ oscillations after insemination. No significant differences were observed in the percentage of oocytes exhibiting Ca2+ oscillations among these groups. However, when the first Ca2+ transient was analysed, differences between SH and the treatment groups were noted. The first peak ratio amplitude in AGX and VPX groups was significantly lower than that of SH (Table II, P < 0.05 and P < 0.01). The relative area of the first peak in AGX, VPX and TX was all significantly smaller than that of SH (Figure 2). Although variability in both amplitude and duration of the oscillations were observed in oocytes inseminated with uterine spermatozoa from AGX and VPX males, the basic pattern of Ca2+ oscillations was similar in most of the oocytes. An exception to this was the TX group, in which 52% of oocytes (13 of 25) showed only one or two and very small Ca2+ transients (Figure 3A), and was significantly higher than that in the SH group (5% or one of 19, P < 0.05, Figure 3B).
**Table II.** Ca\(^{2+}\) oscillations in oocytes induced by epididymal or ejaculated spermatozoa recovered from uteri (mean \(\pm\) SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of eggs examined</th>
<th>No. of eggs showing Ca(^{2+}) transients (%)</th>
<th>No. of sperm fusion in each egg</th>
<th>1st peak ratio (s)</th>
<th>1st peak duration (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH (n = 7)</td>
<td>22</td>
<td>19 (86.4)</td>
<td>2.2 (\pm) 0.3</td>
<td>1.29 (\pm) 0.07</td>
<td>64.8 (\pm) 4.4</td>
</tr>
<tr>
<td>EPI (n = 7)</td>
<td>26</td>
<td>17 (65.4)</td>
<td>2.6 (\pm) 0.4</td>
<td>0.95 (\pm) 0.10(^a)</td>
<td>40.9 (\pm) 2.4</td>
</tr>
<tr>
<td>AGX (n = 7)</td>
<td>24</td>
<td>19 (79.2)</td>
<td>2.1 (\pm) 0.3</td>
<td>0.81 (\pm) 0.11(^a)</td>
<td>58.2 (\pm) 2.7</td>
</tr>
<tr>
<td>VPX (n = 7)</td>
<td>21</td>
<td>19 (90.5)</td>
<td>2.5 (\pm) 0.4</td>
<td>0.64 (\pm) 0.04(^b)</td>
<td>61.7 (\pm) 4.9</td>
</tr>
<tr>
<td>TX (n = 8)</td>
<td>32</td>
<td>25 (78.1)</td>
<td>2.2 (\pm) 0.2</td>
<td>1.04 (\pm) 0.09</td>
<td>57.8 (\pm) 2.5</td>
</tr>
</tbody>
</table>

\(^aP<0.05, ^bP<0.01\) compared with SH; \(^cP<0.001, ^dP<0.01\) compared with EPI.

SH = sham-operated males; EPI = epididymal spermatozoa; AGX = excision of ampullary glands; VPX = excision of ventral prostates; TX = excision of all of major male accessory sex glands; \(n = \) number of males.

**Discussion**

In the present study, we focused on Ca\(^{2+}\) oscillation changes in oocytes inseminated by epididymal and uterine spermatozoa from males with some or all of the accessory sex glands removed. Our results show that male accessory sex gland secretions affect Ca\(^{2+}\) oscillations, shown by the amplitude and duration of the first Ca\(^{2+}\) transient, even though the rate of IVF was not affected (Table I). Furthermore, to the best of our knowledge, this is the first demonstration that Ca\(^{2+}\) oscillations in hamster oocytes inseminated by epididymal and ejaculated spermatozoa recovered from the uterus are different.

In hamsters, the first Ca\(^{2+}\) transient of oocyte after insemination was always higher in amplitude and longer in duration than subsequent ones (Swann, 1990; Miyazaki et al., 1992; Maleszewski et al., 1997). Despite the fact that the pattern of Ca\(^{2+}\) oscillations and frequency was similar in oocytes inseminated with epididymal or uterine spermatozoa (Figure 1A,B), the duration of the first transient in the former group (Table II) was much shorter than those induced by uterine spermatozoa, which suggests that male accessory sex gland secretions have an effect on Ca\(^{2+}\) oscillations. This conclusion is further substantiated by the differences observed between the sham control and treatment groups (Table II). In hamster oocytes a single spermatozoon induces Ca\(^{2+}\) oscillations over a period of 1 h (Miyazaki et al., 1992). In our model, Ca\(^{2+}\) oscillations in most of the oocytes stopped after 30–50 min. This relatively short duration may be due to the bleaching of Fura 2 and/or the absence of protein in the medium.

Epididymal secretory products are known to be essential for mammalian sperm maturation, and evidence indicates that specific proteins become associated with spermatozoa during epididymal transit (Cornwall et al., 1990; Boue et al., 1995, 1996). After epididymal transit, spermatozoa undergo other surface transformations when they encounter male accessory...
Accessory sex glands and oocyte Ca\(^{2+}\) oscillations

The endoplasmic reticulum and leads to Ca\(^{2+}\) oscillations (Shilling et al., 1994; Swann et al., 1998), although such sperm receptors have not yet been fully identified (Tesarik et al., 1994). The other hypothesis is that Ca\(^{2+}\) oscillations are induced by a sperm-derived factor that diffuses into oocytes after the sperm–oocyte membrane fusion (Parrington et al., 1996; Swann et al., 1998). The recent trigger/oscillator hypothesis unifies the oocyte plasma membrane receptor and the sperm factor hypotheses (Tesarik, 1999a). According to this hypothesis, the first Ca\(^{2+}\) transient in the oocyte is induced by the action of the fertilizing spermatozoa at the oocyte plasma membrane just before or during their fusion, whereas the subsequent Ca\(^{2+}\) oscillations are facilitated by a change in the respective sensitivities of the IP\(_3\) and ryanodine receptors, which result from both the first Ca\(^{2+}\) transient and the subsequent action of a soluble spermatozoa cytosolic factor released into the oocyte (Tesarik, 1999b). Based on our present results, we speculate that male accessory sex gland secretions may first affect spermatozoa trigger activity by the modification of sperm plasma membrane and then further influencing subsequent Ca\(^{2+}\) oscillations.

In this study, we also found that Ca\(^{2+}\) oscillations in oocytes induced by epididymal spermatozoa and TX ejaculated spermatozoa were quite different, suggesting that uterine factors also affect this process. Physiological modifications of spermatozoa within the female reproductive tract have been extensively studied (Yanagimachi, 1994). These mainly focused on the effects of oviductal secretions on sperm capacitation, motility and acrosome reaction (Bastias et al., 1993; Zhu et al., 1994; Abe et al., 1995). Little is known about uterine factors on sperm function. During normal fertilization, components from male accessory sex gland secretions such as glycoproteins and fibronectin-like proteins are coated on to the spermatozoa (Koehler et al., 1980; Oliphant et al., 1985). These coated components may prevent spermatozoa from being exposed to uterine secretions. Removal of male accessory sex glands allows uterine factors to act upon spermatozoa. This may partly account for the difference in Ca\(^{2+}\) oscillations of oocytes induced by epididymal spermatozoa and TX ejaculated spermatozoa.

It is well established that Ca\(^{2+}\) oscillations play a central role in the oocyte activation (Kline and Kline, 1994; Bos-Mikich et al., 1995; Maleszewski et al., 1997). In our present study, two different patterns of Ca\(^{2+}\) oscillations induced by uterine sperms are noted, one is oscillatory (Figure 1B) and the other non-oscillatory (Figure 3A). Removal of either ampullary glands or ventral prostates increased the percentage of oocytes with the non-oscillatory pattern, albeit not statistically significantly different compared with the sham control group. After removal of all male accessory sex glands, 52% of oocytes showed the non-oscillatory pattern, whereas oscillatory Ca\(^{2+}\) increases as in normal fertilization, lead to a viable embryo (Sun et al., 1992; Kline and Kline, 1992). Agents that induce oscillatory Ca\(^{2+}\) increases similar to that triggered by the fertilizing spermatozoa prolong greatly the development of the activated oocytes (Ozil, 1990).

Figure 3. (A) Non-oscillatory Ca\(^{2+}\) transients in an oocyte inseminated by uterine spermatozoa recovered from a female mated with a male with all accessory sex glands removed. (B) Summary data of oocytes showing non-oscillatory Ca\(^{2+}\) transients, i.e. only one or two small Ca\(^{2+}\) transients, after insemination of epididymal or ejaculated spermatozoa. *P < 0.05 compared with SH control group. SH = sham-operated males (n = 19); EPI = epididymal spermatozoa (n = 17); AGX = excision of ampullary glands (n = 19); VPX = excision of ventral prostates (n = 19); TX = excision of all of major male accessory sex glands (n = 25).

Sex gland secretions during ejaculation. Modifications of sperm plasma membrane during ejaculation have been poorly defined, and their physiological significance varies from one species to another (Henault et al., 1995). Recent studies suggest that the lower fertilization and pregnancy rates for ICSI with epididymal and/or testicular spermatozoa may be related to a physiological difference in sperm membrane characteristics (Eddy and O’Brien, 1994; Palermo et al., 1996). It may be inferred that mature spermatozoa require some modification so as to promote membrane permeabilization and permit cytosolic sperm factors to find access to the oocyte and produce its activation (Dozortsev et al., 1995; Palermo et al., 1996). In the golden hamster, it has been shown that secretions from ventral prostates and ampullary glands modify sperm plasma membrane proteins, especially glycoproteins (Cheng et al., 1995; Chow et al., 1995). These modifications may later affect Ca\(^{2+}\) oscillations during or after fusion of the sperm and oocyte plasma membrane. The mechanism of oocyte Ca\(^{2+}\) oscillations at fertilization is not fully understood. There are two main hypotheses which explain how the spermatozoon induces Ca\(^{2+}\) oscillations in the oocyte. One is that spermatozoa act via the oocyte plasma membrane receptor to produce inositol 1,4,5-trisphosphate (IP\(_3\)), which releases Ca\(^{2+}\) from the endoplasmic reticulum and leads to Ca\(^{2+}\) oscillations (Shilling et al., 1994; Swann et al., 1998), although such sperm receptors have not yet been fully identified (Tesarik et al., 1994). The other hypothesis is that Ca\(^{2+}\) oscillations are induced by a sperm-derived factor that diffuses into oocytes after the sperm–oocyte membrane fusion (Parrington et al., 1996; Swann et al., 1998). The recent trigger/oscillator hypothesis unifies the oocyte plasma membrane receptor and the sperm factor hypotheses (Tesarik, 1999a). According to this hypothesis, the first Ca\(^{2+}\) transient in the oocyte is induced by the action of the fertilizing spermatozoa at the oocyte plasma membrane just before or during their fusion, whereas the subsequent Ca\(^{2+}\) oscillations are facilitated by a change in the respective sensitivities of the IP\(_3\) and ryanodine receptors, which result from both the first Ca\(^{2+}\) transient and the subsequent action of a soluble spermatozoa cytosolic factor released into the oocyte (Tesarik, 1999b). Based on our present results, we speculate that male accessory sex gland secretions may first affect spermatozoa trigger activity by the modification of sperm plasma membrane and then further influencing subsequent Ca\(^{2+}\) oscillations.
It may be reasonable to assume that even though those oocytes showing non-oscillatory Ca\(^{2+}\) increases can form normal-looking pronuclei, development may be arrested at the time of the maternal–embryonic transition which is a particularly vulnerable period in preimplantation development. In human embryos, it takes place between the 4-cell and 8-cell stage (Braude et al., 1988; Tesarik et al., 1994). This may also be true in the hamster, because we previously found that removal of some or all male accessory sex glands increased the percentage of abnormally developed embryos, which could be detected ultrastructurally at the 4-cell stage (Chow et al., 1994). At the 8-cell stage, the cell number of viable embryos was considerably decreased (O et al., 1988).

The significance of amplitude and duration of Ca\(^{2+}\) transients in the oocyte activation and subsequent embryonic development has not been established. In our present study, we found that some oocytes inseminated by epididymal spermatozoa or uterine spermatozoa from AGX and VPX males showed smaller Ca\(^{2+}\) oscillation amplitude (Table II). Previously we observed that removal of ventral prostates resulted in delayed DNA synthesis in 30–40% of hamster zygotes (Ying et al., 1998); this figure cannot be accounted for entirely by oocytes showing non-oscillatory Ca\(^{2+}\) increases (Figure 3B). We suggest that these attenuated Ca\(^{2+}\) transients may also affect oocyte activation. Sire effects on the Ca\(^{2+}\) transients of oocyte may also explain our other observations of paternal effects on the delayed resumption of meiosis and inactivation of MPF in hamster oocytes during in-vivo fertilization (Ying et al., 1999).

In conclusion, the present findings demonstrate that Ca\(^{2+}\) oscillations of oocytes inseminated by epididymal and uterine spermatozoa are different. Male accessory sex gland secretions affect the amplitude and relative area of the first Ca\(^{2+}\) transient during fertilization. Differences of Ca\(^{2+}\) oscillations induced by epididymal spermatozoa and uterine spermatozoa from males with all accessory sex glands removed suggest that uterine factors may also influence this process. Furthermore, we have demonstrated that ASG has no effect on percentage of zygotes showing two pronuclei (Chow et al., 1994; Ying et al., 1998; Table I), but the fact that Ca\(^{2+}\) oscillations in oocyte activation is abnormal may mean that fertility is compromised.

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Accessory sex glands and oocyte Ca^{2+} oscillations


