Hyperactivation and Acrosome Reaction *In Vitro* in Spermatozoa Ejaculated by Cryptorchid Dogs after Orchiopexy

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ABSTRACT. Orchiopexy of the cryptorchid (CR) testis and castration of the scrotal testis were performed in three unilaterally CR beagles at six months of age. Induction rates for ejaculated sperm hyperactivation (HA) and the acrosome reaction (AR) *in vitro* in these orchiopexied dogs were compared with five those in normal beagles one year later. Canine spermatozoa were incubated for 9 hr at 38°C under 5% CO₂ in air in canine capacitation medium at a concentration of 30×10⁶ sperm/ml. HA was observed using high-speed videomicrography. The AR spermatozoa were evaluated by the triple stain technique. As a result, there was no significant difference between 'the CR dogs after orchiopexy' (CDO) and the normal dogs (ND) with respect to sperm motility just after ejaculation. However, sperm motility of CDO decreased markedly during incubation. There was a significant difference in sperm motility between CDO (Mean ± SD; 47±12%) and ND (80±9%) after three hours of incubation (p<0.01). No significant difference was observed between CDO and ND with respect to the HA rate of motile spermatozoa throughout the incubation period. The peak of HA rate was found in both CDO (58±5%) and ND (61±16%) after seven hours of incubation. The AR rate of spermatozoa in CDO was lower than that in ND after six hours of incubation. The AR rate of CDO (26±4%) was significantly lower than ND (46±5%) after eight hours of incubation (p<0.01). It is assumed that there might be relation between a rapid decrease of motility and low AR rate in spermatozoa of CDO during incubation.—**KEY WORDS:** capacitation, cryptorchidism, dog, spermatozoa.

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The authors have previously reported that orchiopexy in cryptorchid (CR) dogs gives rise to the appearance of spermatozoa in the orchiopexied testes [14]. There is not much difference between spermatozoa produced in the orchiopexied testes and those in normal dog's testes with respect to motility and viability just after semen collection. However, the fertilizing capacity of orchiopexied dogs's spermatozoa was revealed to be quite poor [15]. The mammalian spermatozoa must undergo a process called capacitation consisting of hyperactivation (HA) and acrosome reaction (AR) in order to penetrate eggs [3, 4, 12, 25]. In many species including the dog, sperm HA and AR can be observed by means of experiments *in vivo* and *in vitro* [6, 8, 9, 11, 17, 18, 21, 24]. In the present experiment, induction rates for HA and AR *in vitro* of spermatozoa ejaculated by 'the CR dogs after orchiopexy' (CDO) and the normal dogs (ND) were investigated in order to elucidate the cause of the poor fertilizing ability of spermatozoa of the former.

MATERIALS AND METHODS

Animals and semen collection: Three unilaterally

inguinal CR beagles, six months of age, were subjected to orchiopexy of the CR testis and castration of the scrotal testis by the method previously described [14]. Semen samples were collected weekly by digital manipulation and their quality was tested by the methods previously described [13]. The spermatozoa were incubated *in vitro* one year later when the characteristics of the ejaculate in CDO became stable. Five control beagles of the same age as CDO were examined in the same manner.

Sperm incubation: The medium used for incubation of spermatozoa was canine capacitation medium (CCM) solution (pH 7.8, osmotic pressure 300 m Osm/kg) developed by Mahi and Yanagimachi [18], containing bovine serum albumin 2 mg/ml, penicillin G 100 U/ml, and streptomycin 0.05 mg/ml. A 3 ml of CCM medium was placed in glass test tube (100 × 13 mm) covered with aluminium cap. Semen characteristics were tested immediately after collection and spermatozoa were washed by centrifugation (200 g, 5 min) with CCM medium. The washed spermatozoa were resuspended in the same medium to give a concentration of 30×10⁶ sperm/ml and were incubated for 9 hr at 38°C under

Fig. 1. Motility patterns of incubated dog spermatozoa.

a) These figures show successive positions of a spermatozoon traced at 1/50 sec. intervals.

5% CO₂ in air.

Observation of HA and AR: Sperm HA and AR were checked after 0, 1, 3, 5, 6, 7, 8 and 9 hours of incubation. Sperm movement patterns were recorded by high-speed videomicrography (Nac Co., Ltd., Tokyo, Japan) at 1/50 sec. intervals. Movement pattern A in Fig. 1 was exhibited by all motile spermatozoa just after semen collection. Spermatozoa which showed movement pattern C increased gradually in number throughout the incubation period. According to other reports [5, 19, 26], hyperactivated sperm are considered to exhibit movement pattern C. HA rate of sperm was analyzed by examination of 100 motile sperm which were recorded on a videotape. Live, acrosome-reacted sperm were identified using the triple stain technique described by Talbot and Chacon [22]. Sperm with white heads, except for brown postacrosomal segments, were classified as live and acrosome-reacted and the rate of these sperm was expressed as AR rate.

RESULTS

Semen was collected four times every week from three CDO and five ND. There was no difference in sperm motility between CDO (Mean $\pm$ SD; $86.7 \pm 4.7\%$) and ND ($96.2 \pm 1.5\%$).

Changes in sperm motility during incubation in these CDO and ND are shown in Fig. 2. In ND the mean motility of the incubated spermatozoa more

Fig. 2. The mean ($\pm$ S.D.) rates of motility in spermatozoa during incubation in 3 orchioepexied ($\bullet$) and 5 normal dogs ($\circ$).

**Significant difference from spermatozoa of the normal dogs during incubation ($P < 0.01$).

than 70% was maintained until eight hours of incubation. In CDO, however, the mean motility decreased markedly with increasing incubation time and there was a significant difference in the mean motility between incubated CDO and ND spermatozoa after one hour of incubation ($p < 0.01$). The sperm motilities of CDO and ND after three hours of incubation were $47 \pm 12\%$ and $80 \pm 9\%$, respectively. The HA rates of motile spermatozoa are

Fig. 3. The mean ($\pm$ S.D.) rates of hyperactivation in spermatozoa during incubation in 3 orchiopeXied (—●—) and 5 normal dogs (---○---).

shown in Fig. 3. Changes in the HA rates of incubated spermatozoa in CDO and ND showed similar patterns, and their HA rates were both increased after one hour of incubation. The HA rates of CDO and ND peaked after seven hours of incubation at $58 \pm 5\%$ and $61 \pm 16\%$, respectively.

The AR rates of the incubated CDO and ND spermatozoa are shown in Fig. 4. In spermatozoa ejaculated by CDO the increase in the AR rate disappeared after six hours of incubation and there was a significant difference in the rate between CDO ($26 \pm 4\%$) and ND ($46 \pm 5\%$) after eight hours of incubation ($p < 0.05$).

DISCUSSION

Mahi and Yanagimachi (1978) reported that the active motility and AR rates of ND spermatozoa are 80–90% and 46%, respectively, after seven hours of incubation [18]. In this study, those of ND spermatozoa were $75 \pm 2\%$ and $40 \pm 11\%$, respectively, after seven hours of incubation. The results of the present study are in well accordance with theirs.

It is known that enzymes such as ATPase and minerals such as Ca^{2+} are important to the movement and energy metabolism of spermatozoa [1, 2, 7, 23]. Nagano reported that sperm of the artificial cryptorchid rat has abnormal ultrastructure of the tail [20]. If spermatozoa of CDO have abnormal metabolic systems and/or abnormal ultrastructures in the tail, these sperm motility and viability may remarkably decrease during incubation. It is supposed that such a characteristics of CDO spermatozoa may

Fig. 4. The mean ($\pm$ S.D.) rates of acrosome reaction in spermatozoa during incubation in 3 orchiopeXied (—●—) and 5 normal dogs (---○---).

*Significant difference from spermatozoa of the normal dogs during incubation ($P < 0.05$).

cause the poor fertility. It seems, therefore, necessary to study the ultrastructure of spermatozoa produced by orchiopeXied testes in the future.

As far as the HA rate of motile spermatozoa is concerned, there is not much difference between CDO and ND. It is reported that decapacitation factors are components of seminal plasma [10, 16]. This study revealed that HA of spermatozoa of CDO and ND was similarly induced because of removal of decapacitation factors by washing of their spermatozoa with medium. It is assumed that there might be a relation between a rapid decrease of motility and low AR rate in spermatozoa of CDO during incubation.

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