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Fertilizing Ability of Cock Spermatozoa from the Testis Epididymis and Vas Deferens Following Intramagnal Insemination[1,2]

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ABSTRACT

A reexamination of the fertilizing ability of cock spermatozoa from the testis, epididymis and vas deferens was accomplished through the use of intramagnal insemination. Intramagnal insemination of spermatozoa taken from the testes, epididymides and vas deferentia resulted in fertility levels of 85–90% during the first week and levels of 67–90% the second week after a single insemination. In contrast, vaginal insemination of testicular spermatozoa resulted in a total absence of fertile eggs. Vaginal and intramagnal insemination of ejaculated spermatozoa resulted in fertility levels of 70 and 71%, respectively, during the first week and levels of 42 and 53%, respectively, the second week after a single insemination. There were no significant differences in hatchability of fertile eggs from hens inseminated with semen from different regions of the male tract or by different routes of insemination.

INTRODUCTION

It is well established that mammalian spermatozoa undergo important changes in the epididymis which are necessary for the attainment of functional maturity (Bedford, 1979). Their ability to fertilize eggs appears to be acquired simultaneously with the capacity for motility, while being transported through the epididymis. Both maturation and storage of mammalian spermatozoa appears to depend, moreover, on a special environment created by androgen-dependent activities of the epididymal epithelium.

Considering the fundamental differences that exist between the anatomy and biology of the avian and mammalian reproductive systems, Munro (1935, 1938a,b) investigated the processes underlying functional sperm maturation in the rooster. While withdrawal of testicular androgen reduces drastically the longevity of spermatozoa in the cauda epididymidis in mammals (Orgebin-Crist et al., 1975), the rooster displays no dependence on androgenic support from the testis for storage of spermatozoa in its rudimentary epididymis or vas deferens (Munro, 1938a).

The attainment of the capacity for movement by the formed spermatozoa in the rooster parallels that demonstrated for mammals: capacity for movement is minimal in the testis, increases somewhat in the rudimentary epididymis and reaches maximal ability only in the vas deferens (Munro, 1935, 1938b). Using intravaginal artificial insemination, Munro (1935, 1938b) demonstrated a differential fertilizing ability for sperm from the three regions of the rooster's reproductive tract, the fertilizing ability being directly correlated with power of movement. Thus Munro (1938b) postulated that rooster sperm, like those of mammals, attain a functional maturity during their passage through the epididymis and vas deferens. This "maturation process" which is outwardly manifested in the ability to show movement under proper stimulation, endows them with the capacity to accomplish fertilization.

Since Munro's classical studies, much has been learned concerning sperm transport in the hen's oviduct. Allen and Grigg (1957) demonstrated the importance of sperm motility for traversing the vagina and uterovaginal junction. Allen and Bobr (1955) and Ogasawara et al. (1966) suggested that the uterovaginal glands, as well as the uterovaginal junction, are selective areas of the oviduct preventing sperm characterized by abnormal morphology, poor motility or

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sluggish metabolism from reaching the site of fertilization. Assuming that the minimal motility displayed by testicular and epididymal cock spermatozoa in vitro in Ringer's solution is the same in vivo, Munro's (1935, 1938b) quest to determine their fertilizing ability following intravaginal insemination may have been masked by their minimal capacity for movement, especially in this very selective region of the oviduct.

The purpose of this study, therefore, was to reexamine the fertilizing ability of testicular and epididymal cock spermatozoa through the use of intramagnal insemination, thus bypassing the vagina and uterovaginal junction where sperm motility is essential for transport. The pertinent question is: can testicular and epididymal cock spermatozoa fertilize ova when placed intramagnally in close proximity to the infundibulum or site of fertilization?

MATERIALS AND METHODS

A total of six Arbor Acre broiler-type males were used one per week over a 6-week period. A sample of semen was collected on Monday from the male selected for use that week. Two White Leghorn hens were each artificially inseminated (AI) intravaginally with 0.05 ml of the undiluted semen. The remaining semen was diluted 1:9 (semen:diluent, v/v) with minimum essential medium (MEM, Howarth, 1981). One or two additional hens were then surgically inseminated (SI) intramagnally with 0.1 ml of the diluted semen. The diluted semen was assessed for concentration, motility and dead spermatozoa. The percentage of motile spermatozoa was estimated at room temperature (approx. 25°C). In most types of synthetic diluents, cock spermatozoa are highly active between 20 and 37°C but become reversibly immobilized between 0–5°C and 41–45°C (Munro, 1938c; Ashizawa and Nishiyama, 1978). The sperm concentration was determined with a standard Neubauer hemacytometer after 1:100 dilution in physiological saline with 0.1% formalin added to eliminate motility. The percentage of dead spermatozoa was assessed by the method of Ernst and Ogasawara (1970).

On Wednesday, the male that was used as a semen donor on Monday was sacrificed by cervical dislocation. The body cavity was opened, exposing the reproductive tract, which was carefully excised. Using a scapel, each testis was cut longitudinally in half after separation from the epididymis. Each half was then scored on its medial plane two or three times with the scapel blade. Following this procedure a total of 2.5 to 3.0 ml of fluid exuded from the testes. The exudate was collected and immediately diluted with MEM to a final volume of 5.0 ml. After the epididymis and vas deferens were separated, sperm were stripped from each tissue. Only a small volume of semen (0.05–0.10 ml) could be obtained from the epididymis and this was diluted to a final volume of 1.0 ml with MEM. The vas deferens semen yield ranged between 0.8 and 1.0 ml and this was diluted to a final volume of 10.0 ml with MEM.

Due to the rather short fertilizable life span of undiluted ejaculated cock spermatozoa in vitro at temperatures between 20 and 40°C (Garren and Shaffner, 1952), and the length of time (approx. 2–2.25 h) from killing the semen donor to surgically inseminating the last hen, we chose to dilute the semen immediately upon collection to minimize drying and to preserve viability. The procedure used for holding semen (Howarth, 1981) is capable of maintaining the viability of cock semen in vitro for 6 h at 41°C. Based upon initial observations on numbers of spermatozoa likely to be recovered from the various regions of the male tract, we chose a constant volume of diluted semen to inseminate into each region that would provide approximately 25% of the sperm numbers that would normally be inseminated intravaginally.

Surgically inseminated hens were first anesthetized by intravenous injection of 0.1 ml of a mixture of xylazine (20 mg/ml):ketamine hydrochloride (100

TABLE 1. Evaluation of semen from different regions of the male reproductive tract.

Semen group	No. of sperm X 10^8 inseminated Mean (range)	Volume inseminated (ml)	Mean ± SEM Semen evaluation motile (%)	dead (%)
Testicular				
AI-vagina	1.2 (1.0–1.6)	.50	19 ± 9[c]	7 ± 3[a]
SI-magnum	1.0 (0.9–1.5)	.45	19 ± 9[c]	7 ± 3[a]
Epididymal				
SI-magnum	2.1 (0.4–2.8)	.30	49 ± 25[b]	7 ± 2[a]
Vas Deferens				
SI-magnum	0.7 (0.4–1.0)	.10	88 ± 4[a]	3 ± 1[b]
Ejaculated				
AI-vagina	3.1 (1.4–4.9)	.05	87 ± 10[a]	4 ± 2[b]
SI-magnum	0.6 (0.3–1.0)	.10	87 ± 10[a]	4 ± 2[b]

[a,b,c]Values within a column not having one letter in common differ significantly ($P<0.05$).

mg/ml), (1:1, v/v). An incision was made on the left side of the hen immediately anterior to the thigh. Diluted semen obtained from the testis, epididymis or vas deferens was inseminated into the magnum in volumes of 0.45, 0.30, and 0.10 ml, respectively, using a 20-gauge needle attached to a tuberculin syringe. Our intent was to have two hens for each semen source per male used. This was not always possible due to complications encountered during surgery and the failure of some to lay following surgery. During the last half of the experiment, an additional two hens per week were intravaginally (nonsurgically) inseminated with 0.50 ml of diluted testicular spermatozoa. Immediately following insemination, the diluted semen was assessed for concentration, motility, and dead spermatozoa as previously described. Following this single insemination, fertility and hatchability were evaluated for eggs laid over a 2-week period. Eggs were collected daily, beginning the second day after artificial or surgical insemination, set weekly, and candled after 5 days of incubation. Eggs thought to be infertile were broken open and examined macroscopically for evidence of embryonic development. Fertile eggs were incubated to term to determine hatchability.

Results were evaluated by analysis of variance (ANOVA) and differences between treatment means were analyzed by Duncan's multiple range test using the ANOVA procedure in SAS (Helwig and Council, 1979).

RESULTS

Coinciding with Munro's (1938b) observations, the percentage of motile spermatozoa was low for samples of testicular cells, but was higher for samples from the epididymis and especially the vas deferens (Table 1). The percentage of dead spermatozoa, which ranged from 3 to 9%, was higher in samples from the testis or epididymis than in samples from the vas deferens or ejaculated semen.

In evaluating the percentage of dead testicular spermatozoa by the method of Ernst and Ogasawara (1970), we simultaneously obtained counts of anatomically immature sperm cells (i.e., cells which had not completed spermiogenesis). Between 25 to 30% of the testicular spermatozoa surgically or artificially inseminated were classified as anatomically immature cells.

Fertility results for cock spermatozoa obtained from different regions of the male tract are summarized in Table 2. Following surgical insemination, seven hens (17%) either failed to lay or laid only one egg within 24 h of the surgery and were not included in the data presented in Table 2.

Testicular spermatozoa were infertile when inseminated into the vagina, but were highly fertile when inseminated into the magnum. Through the first week following a single insemination, fertility of spermatozoa from the testis, epididymis and vas deferens was not significantly different and was similar to that obtained with ejaculated spermatozoa inseminated into the magnum. There was no significant differences in hatchability of fertile eggs from hens inseminated with semen from different regions of the male tract or by different routes of insemination.

DISCUSSION

It can be clearly seen that testicular spermatozoa, when deposited in the vagina, produced zero fertility. However, when aliquots of these samples of testicular spermatozoa were surgically deposited in the magnum, a high level of fertility was obtained. Based upon these observations, I suggest that testicular spermatozoa, when placed within the magnum, can rapidly attain the capacity for movement and proceed to fertilize ova. In view of the brief time required for their epididymal passage (de Reviers, 1975), the attainment of the capacity for movement must be acquired within a few hours. Assuming the necessity for sperm motility in the fertilization process, the attainment of functional maturity in this case would not be restricted to the male tract but could be accomplished in the oviduct (magnum) as well.

If, on the other hand, active movement of spermatozoa is unnecessary for fertilization, such a possibility being alluded to by Allen and Grigg (1957), then my results reveal that testicular spermatozoa are fully capable of fertilizing ova upon release from the testis, assuming they are deposited above the uterovaginal junction. Motility, in this case, would only be required for transport through the vagina and uterovaginal junction (Allen and Bobr, 1955; Allen and Grigg, 1957; and Ogasawara et al., 1966).

Another explanation of the results might be that a limited number of spermatozoa do, in fact, attain full functional maturation in the testis. Studied carefully, the early results of Munro (1938b) show that some cock spermatozoa can mature to a fertile state within the testis. By virtue of being surgically deposited in the magnum (as opposed to the vagina), in close proximity to the site of fertilization and the infundibular sperm storage sites, those mature spermatozoa may be sufficient in number to insure the fertilization of successively ovulated ova.

While the results of this study indicate that

TABLE 2. Fertility and hatchability following insemination with semen from different regions of the male reproductive tract.

Semen group	No. of hens	1st Week			2nd Week		
		No. of eggs set	Fertility (%)	Hatchability of fertile eggs (%)	No. of eggs set	Fertility (%)	Hatchability of fertile eggs (%)
Testicular							
AI-vagina	6	33	0 ± 0[b*]	...	21	0 ± 0[c]	...
SI-magnum	11	53	85 ± 8[a]	98 ± 2[a]	48	67 ± 11[ab]	96 ± 3[a]
Epididymal							
SI-magnum	7	35	86 ± 14[a]	94 ± 4[a]	30	90 ± 5[a]	88 ± 9[a]
Vas Deferens							
SI-magnum	9	47	90 ± 8[a]	82 ± 11[a]	36	77 ± 12[ab]	90 ± 5[a]
Ejaculated							
AI-vagina	9	48	70 ± 13[a]	88 ± 7[a]	43	42 ± 11[b]	89 ± 8[a]
SI-magnum	7	35	71 ± 14[a]	97 ± 3[a]	31	53 ± 13[ab]	91 ± 6[a]

[a,b,c]Values within a column not having one letter in common differ significantly ($P<0.05$).

*Mean percentage ± SEM.

testicular spermatozoa can fertilize ova when deposited intramagnally, it raises additional questions concerning maturation and the acquisition of motility by testicular spermatozoa in the oviduct and need for motility above the uterovaginal junction. These questions will require further investigation.

The low fertility obtained following intravaginal insemination of ejaculated semen can most likely be explained by the time of day the hens were inseminated, which routinely occurred between 1000 and 1100 h. Johnston and Parker (1970) have indicated that fertility is decreased when hens are artificially inseminated within 1 h after or 4 h before oviposition.

In marked contrast to these observations, mammalian spermatozoa taken from the testis or the proximal part of the ductus epididymidis are largely or totally infertile. Spermatozoa collected by catheter from the rete testis of rams were infertile even though spermatozoa could be recovered from the fallopian tubes after insemination (Setchell et al., 1969). Bedford (1966) reported that when spermatozoa from specific segments of the rabbit epididymis were inseminated intratubally 10 h before ovulation, virtually no ova were fertilized by sperm samples taken from the regions proximal to the middle of the corpus epididymidis. Sperm from the caput epididymidis and the proximal half of the corpus region not only fail to fertilize, but are also apparently unable to establish contact with the surface of the zona pellucida, indicating a specific reliance on epididymal transit for the acquisition of fertilizing ability.

REFERENCES

Allen, T. E. and Bobr, L. W. (1955). The fertility of fowl spermatozoa in glycerol diluents after intrauterine insemination. Poult. Sci. 34: 1167–1169.

Allen, T. E. and Grigg, G. W. (1957). Sperm transport in the fowl. Aust. J. Agric. Res. 8:788–799.

Ashizawa, K. and Nishiyama, H. (1978). Effects of temperature on the vigour of motility, oxygen consumption and duration of motility of fowl spermatozoa under aeorbic conditions. Jpn. Poult. Sci. 15:264–266.

Bedford, J. M. (1966). Development of the fertilizing ability of spermatozoa in the epididymis of the rabbit. J. Exp. Zool. 163:312–329.

Bedford, J. M. (1979). Evolution of the sperm maturation and sperm storage functions of the epididymis. In: The Spermatozoon, Maturation, Motility, Surface Properties and Comparative Aspects (D. W. Fawcett and J. M. Bedford, eds.). Urban and Schwarzenberg, Baltimore-Munich, pp. 7–21.

de Reviers, M. (1975). Sperm transport and survival in male birds. In: The Biology of Spermatozoa, INSERM, Nouzilly (E.S.E. Hafez and C. G. Thebault, eds.). S Karger, Basel.

Ernst, R. A. and Ogasawara, F. X. (1970). A live-dead stain to test poultry semen quality. Univ. California Agr. Ext. Ser., OSA #n193.

Garren, H. W. and Shaffner, C. S. (1952). The effect of temperature and time of storage on the fertilizing capacity of undiluted fowl semen. Poult. Sci. 31:137–145.

Helwig, J. T. and Council, K. A., eds. (1979). SAS User's Guide. SAS Institute, Inc., Raleigh, NC.

Howarth, B, Jr. (1981). Preservation of the fertilizing capacity of cock semen incubated *in vitro* at 41 C. Poult. Sci. 60:1075–1078.

Johnston, N. P. and Parker, J. E. (1970). The effect of time of oviposition in relation to insemination on fertility of chicken hens. Poult. Sci. 49:325–327.

Munro, S. S. (1935). Motility and fertilizing capacities of fowl sperm in the excretory ducts. Proc. Soc. Exp. Biol. Med. 33:255–257.

Munro, S. S. (1938a). The effect of testis hormone on the preservation of sperm life in the vas deferens of the fowl. J. Exp. Biol. 15:186–196.

Munro, S. S. (1938b). Functional changes in fowl sperm during their passage through the excurrent ducts of the male. J. Exp. Zool. 79:71–92.

Munro, S. S. (1938c). Fowl sperm immobilization by a temperature-media interaction and its biological significance. Q. J. Exp. Physiol. Cogn. Med. Sci. 27:281–291.

Ogasawara, F. X., Lorenz, F. W. and Bobr, L. W. (1966). Distribution of spermatozoa in the oviduct and fertility in domestic birds. III. Intrauterine insemination of semen from low-fecundity cocks. J. Reprod. Fertil. 11:33–41.

Orgebin-Crist, M. C., Danzo, B. J. and Davies, J. (1975). Endocrine control of the development and maintenance of sperm fertilizing ability in the epididymis. In: Handbook of Physiology: Endocrinology, vol. 5 (R. O. Greep and D. W. Hamilton, eds.). American Physiological Society, Bethesada, MD, pp. 319–338.

Setchell, B. P., Scott, T. W., Voglmayr, J. K. and Waites, G.M.H. (1969). Characteristics of testicular spermatozoa and the fluid which transports them into the epididymis. Biol. Reprod. 1 (Suppl. 1):40–66.