Sperm Function Assays

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Abstract

Evaluation of sperm function provides finality to the battery of in vitro assays while discriminating between a good from that of bad quality semen sample. In this important chapter, we have described zona binding assay with four types of variations, namely, hemizona, zona free, heterologous and homologous zona binding assays. Merits and demerits of these assays have been discussed here.

Keywords

Zona binding • Hemizona • Homologous • Heterologous • In Vitro fertilization

Contents

20.1 Introduction

Selection of sire with a high fertility is economically important from animal production point of view. Successful fertilization depends on quality of semen. As male infertility is a multifactorial disorder, sometimes good-quality semen also does not result in acceptable fertility [1]. Although artificial insemination (AI) is a reliable method for evaluation of male fertility, it is time-consuming as well as expensive. Hence, determination of male fertility by in vitro fertilization

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technique would be cost and time saving compared to field fertility tests [2–4]. Fertilization is the result of precisely ordered sequences of cellular interactive processes starting from interaction of gametes in oviduct [6]. After traversing through cumulus cells, sperms encounter the zona pellucida and undergo zona regulated acrosomal exocytosis to move through this proteinous barrier to form the pronucleus [7–9]. Abnormality in either sperm or oocyte leads to failure of attachment of sperm with the zona layer of oocytes [10]. Many in vitro assay protocols have been developed for better understanding of fertilization and accurate prediction of male fertility.

A battery of sperm function tests provide useful insights in predicting male fertility than any single test adopted for finding out the exact reason for fertilization failure [11]. In vitro tests like zona binding assay (ZBA), hyaluronic binding assay (HBA) and in vitro fertilization (IVF) are not only interpret the interaction between gametes of opposite sexes but also deduce the exact reason for fertilization failure. ZBA has been used to predict male fertility in humans and various domestic animals like ox, dog and cat [12–14]. The test has also been used to identify the molecular defects in sperm function [3, 15] (Fig. 20.1).

20.2 Comparative Merits of Various Zona Binding Assays

The current chapter describes four types of zona binding assays as detailed below:

- Hemizona binding assay
- Zona-free hamster oocyte penetration assay
- Homologous sperm-zona pellucida assay
- Heterologous sperm-zona pellucida assay (Table 20.1)

20.3 Hemizona Binding Assay

Two different types of sperm-ZP binding assay have been developed; Zhang et al. [17] used intact (not cleaved) homologous oocytes, and Fazeil et al. [18] used bisected hemizonae (hemizona binding assay), where each half incubated with standard and test spermatozoa, respectively. The hemizona binding assay (HZA) measures the binding of spermatozoa to internal as well as external surfaces of zona pellucida (Fig. 20.2).

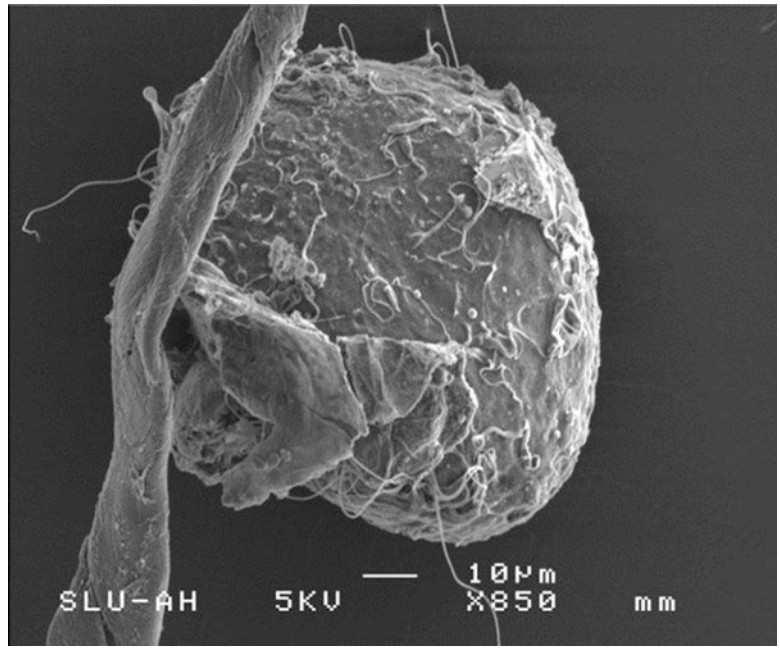

Fig. 20.1 Scanning electron micrograph of spermatozoa bound to zona pellucida of a frozen-thawed feline oocyte [16]

Table 20.1 Comparative merits and demerits of zona binding assays

Zona binding assays	Advantages	Disadvantages
Hemizona binding assay	As each half of oocytes (hemizona) have equal surface, it thus allows controlled comparison	Limited availability of zona sperm receptors
	As oocytes are splitted manually, those will not be fertilized by any of the binding spermatozoa	
	Assay is reproducible	
Zona-free hamster oocyte penetration assay	Zona-free hamster eggs allow entry of capacitated spermatozoa of a wide variety of heterologous species and hence provide a reliable sperm penetration assay	The usage of zona-free hamster egg penetration test is limited due to test's complexity, cost involvement and difficulty in its standardization
	Hamster oocyte lacks distinct membrane to block polyspermy; hence a large number of spermatozoa penetrate to constitute fertilization [5]	Interassay variability is the main drawback of this assay method
		The variation between individual oocytes in sperm-binding capacity is the major drawback of this assay
		Frozen-thawed ovaries exhibited reduced sperm-binding capacity compared to that of freshly collected oocyte
Homologous zona binding assay	The sperm in vitro binding assay in homologous oocytes is useful to estimate the fertility potential	The variation between individual oocytes in sperm-binding capacity is the major drawback of this assay
		Frozen-thawed oocytes exhibit reduced sperm-binding capacity compared to that of freshly collected oocytes

Applications of Hemizona Binding Assay

(a) To assess sperm fertilizing ability of domestic animals.
(b) Zona pellucida binding index can be determined using zona binding capacities of test and control semen samples.
(c) Assessment of sperm concentration required for fertilization in case of abnormal morphology [19].
(d) HZA may be used to predict the effects of semen treatment and preservation methods on their fertilizing capacity, to screen the donors as well as to assess insemination doses [20].
(e) HZA helps in analysis of physiological and cellular events happening prior to fertilization [10].

20.3.1 Hemizona Assay (Human)

The protocol describes the HZA for human [21].

Oocyte Collection

(a) Ovaries collected by ovariohysterectomy or from slaughter house can be used for harvesting of oocytes.
(b) Wash the ovaries with PBS, and cut into 2–3 pieces. Immerse the ovarian pieces in PBS supplemented with 2% DMSO and equilibrate for 1 h at room temperature and subsequently for 1 more hour at 5 °C before freezing at −18 °C for less than 1 month. After completion of freezing period, thaw the pieces of ovaries for 5 min in a water bath at 50 °C. Then mince the ovarian tissue mechanically and harvest the oocytes under a stereo zoom microscope.
(c) Wash the harvested oocytes 4 times with PBS to separate the surrounding granulosa cells, and rinse the denuded oocytes 4 times with cryoprotectant medium.
(d) Load the oocytes along with little quantity of freezing medium in 0.12 ml straw, and keep at 5 °C for 1 h. Before storing the straws in

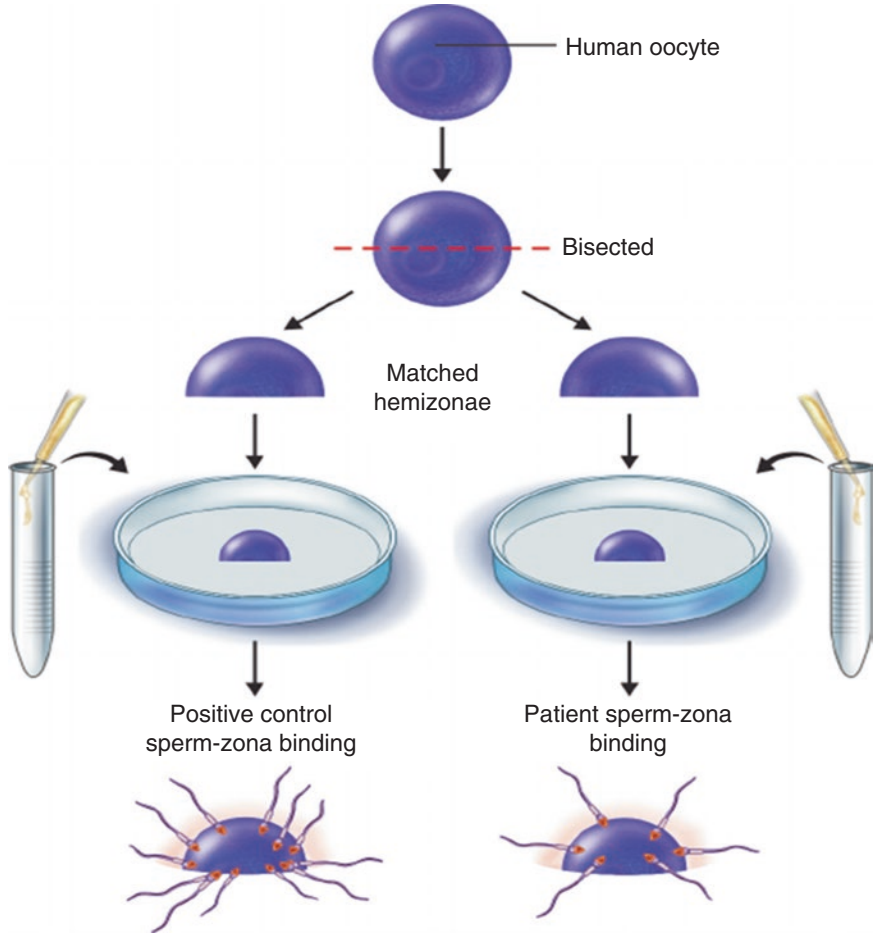

Fig. 20.2 Schematic representation of hemizona binding assay

liquid nitrogen, suspend them 5 cm above liquid nitrogen for 15 min. Cryopreserve the straws till use.

(e) At the time of experiment, thaw the straws in a water bath at 37 °C for 8 sec.

(f) Pour the content of straw into a glass petri dish containing 1 ml PBS. Wash the oocytes thrice by transferring them serially into PBS droplets (20 µl). Discard the oocytes with defective zona later. Then put the oocytes in a 10 µl droplet of fertilization medium.

(g) Section the oocytes into two equal halves using fine needles inside the droplet of fertilizing medium.

(h) Drag the dense ooplasm present inside the sectioned zona towards the centre of droplet using needle or suck it using a mouth pipette.

(i) Then transfer the hemizonae into a 50 µl droplet of fertilization medium, and use those in HZA within 18–24 h.

Sperm Preparation

(a) Sperm motility, capacitation and acrosome reaction are important events to achieve successful fertilization. Therefore, semen parameters like sperm motility and concentration and percentage of live/dead sperm must be evaluated prior to sperm preparation for HZA.

(b) Capacitate the spermatozoa by incubating diluted fresh/frozen-thawed semen sample with 2 ml of fertilization medium (in a 12 × 22 mm round tube) in a water bath at

37 °C for 1 h. After 1 h of incubation, harvest 1 ml of the supernatant-containing spermatozoa, and incubate it again at 37 °C in a water bath.

(c) After completion of incubation, evaluate the sperm motility and concentration of the semen sample. Then dilute the capacitated semen sample as per requirement of experiment (HZA).

Procedure

(a) Incubate the petri dish containing hemizonae in droplet at 37 °C for 2 h.
(b) Then add 100 µl of capacitated-diluted semen sample to the droplet containing hemizona, and incubate at 37 °C.
(c) Wash the hemizona twice in 20 µl droplets of PBS after 1 h of co-incubation with spermatozoa to remove loosely attached sperms.
(d) Put each hemizona in a droplet of 5% methanol-eosin or 2% PFA on a slide. Put a coverslip over the hemizona, and apply a thread of Vaseline to the edge of coverslip to prevent evaporation.
(e) Count the number of spermatozoa attached to outer surface of hemizona under inverted microscope at 400X magnification.

20.3.2 Hemizona Assay (Bovine)

This section describes hemizona binding assay for bulls [18].

Semen Analysis and Cryopreservation

(a) Collect the ejaculates from mature bull through artificial vagina (AV), and process for freezing.
(b) Assess mass motility under a light microscope at 100X magnification.
(c) Determine the percentage of live-dead as well as abnormal spermatozoa after staining the spermatozoa with eosin-nigrosin stain under oil immersion lens of a light microscope.
(d) Determine the sperm concentration using haemocytometer, and then dilute the semen sample with Tris-egg yolk-glycerol extender with a final concentration of 20×10^6 sperms/ml.
(e) Fill the semen in straws (0.25 ml capacity) and equilibrate at 5 °C for 4 h before cryopreservation.
(f) Cryopreserve the semen straws in liquid N_2 using a freezing processor (cooling rate, 60 °C/min).
(g) Fresh ejaculate displaying more than 70% and cryopreserved thawed semen with at least 45% motility should be used as control.

Oocyte and Hemizona Preparation

(a) Ovaries collected from slaughter house can be used to harvest oocytes.
(b) Collected ovaries must be transported to laboratory within 2 h of slaughter in NSS/PBS fortified with gentamicin at 50 µl/ml.
(c) Aspirate oocytes from non-atretic surface follicles with 18 gauge needle attached to a 5 ml syringe containing oocyte collection medium. Pool the cumulus oocyte complex and follicular fluid into a 50 ml sterile tube and allow setting for 10 min in a BOD incubator at 37 °C. Discard the upper two thirds supernatant and pour the sediment in a 90 mm petri dish. Search the oocytes under stereo zoom microscope, and pick them with the help of a mouth pipette into OCM droplets. Remove the cumulus cells by repeatedly washing them in OCM droplets.
(d) Wash the cumulus-free oocytes with PBS and transfer them into 50 µl droplets of PBS (15–20 oocytes/droplet) in a 35 mm plastic petri dish.
(e) Bisect the oocytes at midline under inverted phase contrast microscope using micromanipulator or fine needle.
(f) Remove the dense ooplasm present inside each hemizonae with help of microneedle.
(g) Put hemizonae in different droplets (50 µl) of fertilization TALP medium in a 35 mm petri dish. Cover the droplets with mineral oil at 4 °C till use in HZA.

Sperm Preparation

(a) Thaw straws containing test and control semen sample at 37 °C for 30 sec.
(b) Dilute the semen samples with PBS up to 5 ml.
(c) Wash the semen samples twice with PBS by centrifuging for 10 min at 600 g. Wash the semen samples again with TALP following the above procedure. Reconstitute the washed sperm pellet with freshly prepared fertilization TALP.
(d) Assess the motility of sperms and resuspend in the fertilization TALP at concentration of 1×10^5 motile sperms/ml.

Procedure

(a) Add 50 µl of sperm suspension to droplet containing hemizona, and incubate in a CO_2 incubator at 37 °C, 5% CO_2 and fully humidified atmosphere for 4 h.
(b) Wash the sperm-hemizona complex gently with PBS several times to remove loosely attached sperms using a mouth pipette whose inner diameter is larger than the size of the complex.
(c) Fix the sperm-hemizona complex with PBS supplemented with 2.5% glutaraldehyde for 10 min. Then stain the complex with Hoechst 33,342 dye (10 µg/ml) after washing the complex with PBS thrice.
(d) Count the number of spermatozoa bound to hemizona under a fluorescent microscope. Again to avoid discrimination between spermatozoa bound to inner and outer surface of hemizona, counting must be done under a stereo zoom microscope.
(e) The number of spermatozoa bound to the outer surface of the hemizona (N) was calculated by the formula: $N = b \times a / b + c$, where 'a' is the total number of spermatozoa bound to a hemizona, 'b' is the number of spermatozoa bound to the outer surface of the hemizona and 'c' are the number of spermatozoa bound to the inner surface of the hemizona.

Inference

$$\text{Hemizona Index} = \frac{\text{No. of sperm bound to the outer side of a test hemizona}}{\text{No. of sperm bound to the outer side of a control hemizona.}}$$

Critical Parameters in Hemizona Assay

Non-inseminated oocytes bind with more number of spermatozoa compared to unfertilized oocytes from IVF. Similarly, oocytes harvested from preovulatory follicle bind with higher number of spermatozoa than immature oocytes [22].

Chemical Composition of Media

Spermatozoa incubated in EBSS (Earle's Balanced Salt Solution) exhibit higher zona binding capacity than spermatozoa incubated in Ham's F-10 medium. This may be due to higher Ca concentration of EBSS than Ham's F-10 medium [7].

Protein Supplementation

The quality of supplemented protein in maturation medium and TALP or presence of certain chemical affects the hemizona assay.

Diameter of the Pipette

The diameter of the oocytes is approximately 150 µm. So the inner diameter of pipette used to remove loosely bound spermatozoa to hemizona should not be less than 200 µm in order to prevent the removal of tightly bound spermatozoa [23].

20.3.3 Background Information

(a) The capacity of ZP to bind spermatozoa remains unchanged after either cryopreservation in liquid N_2 with DMSO as cryoprotectant or preserved in salt solution at 4 °C [24]. Prolonged preservation (more than a year) at 4 °C significantly lowers the ZP's sperm binding capacity [22].
(b) Oocytes harvested from preovulatory follicle bind to higher number of spermatozoa compared to immature oocytes [25].
(c) Sperm binding to ZP is influenced by factors like chemical composition of medium used, supplemented proteins in base medium and the size of pipette used to dislodge loosely bound spermatozoa [26].
(d) In vitro capacitation and acrosome reaction are influenced by the type of protein supplemented in base medium.
(e) Same medium should be used for both HZA and IVF to predict IVF accurately through HZA for a particular laboratory [23].
(f) Diameter of the pipette used to dislodge the loosely bound spermatozoa should not be more than 200 μm [23].

20.4 Zona-Free Hamster Oocyte Penetration Test

Zona-free hamster oocyte penetration test is one of the best sperm penetration assays for evaluating sperm fertilizing ability. This test indicates the penetration power of spermatozoa into the zona-free hamster egg. Zona-free hamster eggs are used to assess the sperm penetration capability of a wide range of heterologous species of animals including humans as they allow entry of capacitated sperms of all most all species of animals [27, 28]. Xenogenic sperm penetration assay using zona-free hamster oocyte consists of preparation of spermatozoa and zona-free hamster oocytes and co-incubation of sperm-oocyte.

Application

(a) This is a laboratory test that accurately evaluates fertilizing capability of spermatozoa of different species of animals using hamster eggs [29].
(b) Zona-free hamster eggs allow xenogenic sperms with great ease as its zona have been removed prior to co-incubation with sperms [30].

Procedure

(a) Carefully transfer the released eggs to a sterilized petri dish (35 mm) containing sperm TALP medium using a mouth pipette.
(b) Remove the cumulus cells surrounding oocytes by washing repeatedly with TALP medium using a mouth pipette.
(c) Transfer the eggs to 0.1% trypsin solution for 1 min.

Transfer hamster oocytes from trypsin solution to TALP immediately after dissolution of their zona layer.

(d) Wash the zona-free oocytes thrice with TALP before transferring them into fertilization medium for co-incubation with sperms.
(e) Transfer 8–10 zona-free hamster ova into sperm suspension drop (100 μl) (sperm concentration, 3–6 million/ml).
(f) Cover the drops containing sperm and oocytes with mineral oil, and incubate in a CO_2 incubator at 39 °C with a 5% CO_2 level and 99% humidity for 3 h.
(g) Remove the ova from drop of sperm suspension and wash for four times with medium.
(h) Put the ova along with small amount of medium at centre of four wax spots on a glass slide to evaluate penetrability of spermatozoa.
(i) Put a coverslip over sample and press gently to flatten the ova without rupturing it.
(j) Examine the under 400X magnification to evaluate the sperm penetrability.

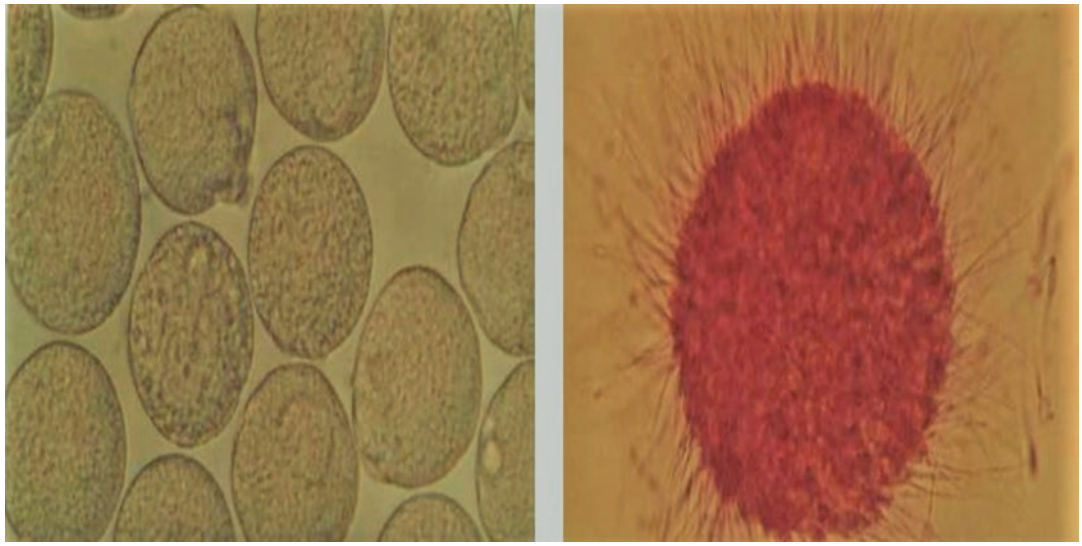

Fig. 20.3 Microphotograph shows zona-free hamster ova (ZFHO; 320 X) and buffalo sperm interacting with ZFHO (400 X) [33]

(k) Ten percent of the penetrated oocytes each having five spermatozoa are considered being normal value [31]
(l) Evaluation should be done considering the number of spermatozoa connected to oolemma [32] (Fig. 20.3).

Points to Ponder

(a) Capacitated spermatozoa that have undergone the process of acrosome reaction can only be able to penetrate zona-free hamster oocytes.
(b) Capacitation of spermatozoa can be achieved by incubating them in a capacitation inducing medium for 1 h. Ca ionophores, follicular fluid, glycosaminoglycans, egg-yolk buffer, platelet activation factor, progesterone, etc. are normally used to induce in vitro capacitation and acrosome reaction.
(c) For better visualization under microscope, the oocytes can be stained with eosin stain (1%) before mounting.

20.4.1 Background Information

Colonies of hamsters used for harvesting eggs should be provided with ad libitum high energy content food and clean water. Hamster sheds should be cleaned daily with disinfectants. Their cages and litter material should be changed every 4–5-day interval. Provision of fresh drinking water twice daily is a must for their health. They require 11–12 h of light in their shed for breeding purpose. Female hamster ovulates 8–16 eggs after LH surge during the estrous cycle. More number of eggs can be harvested from female hamsters by adopting superovulation procedure.

20.5 Homologous Sperm-Zona Pellucida Binding Assay

A number of laboratory tests based on capacity of spermatozoa to bind to homologous zona pellucida have been used to evaluate accurately the

sperm fertilizing ability in different animal species [34].Sperm must bind and penetrate zona pellucida to reach and fertilize oocyte. During the fertilization process a large number of sperms reach the zona pellucida, but only one of them is able to penetrate the zona to fertilize the egg. After that all other sperms remain attached to the zona due to 'zona reaction', and they are called as accessory sperms. Accessory sperms are accepted as a measure of sperm transportation and competition to fertilize oocyte. The number of accessory sperms trapped in zona layer reported to be positively co-related with the fertility.

Oocyte Recovery and Selection

(a) Collect the ovaries from slaughter for harvesting oocytes out of them.
(b) Transport collected ovaries in normal saline fortified with antibiotics (gentamicin at 50 μg/ml) to laboratory within 2 h of slaughter.
(c) Remove the tissues attached to surface of ovaries, and wash them with NSS.
(d) Aspirate the oocytes from surface follicles of 2–5 mm size using 18 gauge needle attached to 5 ml syringe.
(e) Collect the oocytes with 2–3 layers of cumulus cells and homogenous cytoplasm using a mouth pipette under stereo zoom microscope.

Oocyte Maturation

(a) Wash the selected oocytes thrice with oocytes maturation medium.
(b) Incubate the oocytes in maturation medium droplets covered with mineral oil in a CO2 incubator at 38.5 0C, 5% CO2 and 95% humidity for 27 h.
(c) After maturation remove the cumulus cells by repeated washing in the medium.
(d) Transfer the denuded oocytes to fertilization TALP droplets (one oocyte per 50 μl droplet).
(e) Preserve the oocytes in droplet at 4 0C after covering the droplets with mineral oil till use in the experiment.

Semen Analysis and Cryopreservation

(a) Collect the semen ejaculates from mature bull through artificial vagina (AV).
(b) Assess the mass motility of spermatozoa at 100X magnification.
(c) Determine the sperm morphology and live/dead count after staining with eosin-nigrosin stain under oil immersion objective of a microscope.
(d) Determine the sperm concentration using a haemocytometer, and dilute accordingly using TALP.
(e) Fill the straws (0.25 ml volume) with semen and cryopreserve in liquid nitrogen till use.
(f) Fresh ejaculates displaying more than 70% and cryopreserved thawed semen with at least 45% motility should be used as control.

Sperm Preparation

(a) Thaw frozen test and control semen samples (four straws each) and pool them separately.
(b) Adjust volume of the pooled semen samples to 5 ml with PBS.
(c) Wash the sperms twice with PBS and once with sperm TALP by centrifuging at 600 g for 10 min.
(d) After assessing sperm motility, resuspend sperms in sperm TALP at 10×10^6 motile sperm/ml.

Procedure

(a) Co-incubate the sperm and oocyte in a CO2 incubator for 4 h at 38.5 0 C, 5% CO2 and 95% humidity, after adding 50 μl of sperm suspension to each oocyte droplet.
(b) Wash the sperm-oocyte complexes on completion of incubation period to remove loosely attached spermatozoa.
(c) Fix the sperm-oocyte complex with 2.5% glutaraldehyde in PBS for 10 min and then wash with PBS.
(d) Stain the complexes with Hoechst 33,342 dye (10 μg/ml) for 10 min. Wash with PBS and count the number of attached spermatozoa to oocyte using a fluorescent microscope at 200 X magnification.

Interpretation

Collect oocytes from ovaries obtained from animals slaughtered at same time, and divide them into two groups.

Compare the zona pellucida binding capacity of test and control semen samples by incubating them with different groups of oocytes.

$$\text{Zona binding Index} = \frac{\text{No. of sperm bound to the test group oocytes}}{\text{No. of sperm bound to the control group oocytes}}$$

Calculate the zona binding index of each test semen sample, and compare with that of control semen sample.

Points to Ponder

(a) Use of intact oocyte in homologous sperm-zona pellucida assay makes it simpler than hemizona assay or zona-free hamster penetration assay. Again the use of more number of oocyte at same time minimizes the variability [17].
(b) Zona binding assay reflects different aspects of semen quality like sperm morphology, motility, presence of intact acrosome and capacity to penetrate oocytes [34].
(c) Zona pellucida of immature oocytes differs from those mature oocytes in different glycoprotein contents (Zp1, Zp2 and Zp3) as well as in number of sperm receptors. Zona of mature oocytes contain more receptors than zona of immature ones.
(d) Low penetration rate in immature oocytes is mostly due to difference in thickness, composition and number of sperm receptors of immature zona pellucida.
(e) The number of spermatozoa from a control semen sample bound to an oocyte zona layer should be taken as standard to predict the zona binding capacity of test semen samples.

Troubleshooting

(a) The number of spermatozoa bound to zona of an oocyte in the test does not represent the actual zona binding capacity of the tested semen sample.
(b) Interassay variability in binding to number of spermatozoa by oocytes is a major drawback of the test.
(c) The assay cannot predict male fertility accurately as some abnormal sperm without oocyte penetration capability are able to bind to zona pellucida.
(d) Non-availability of freshly harvested oocyte of certain species of animals is a major hindrance to the assay procedure, as preserved oocytes have a reduced sperm binding capacity.

20.5.1 Background Information

(a) Successful fertilization depends on effective binding between spermatozoa and zona pellucida of oocyte. Defective association of sperm with zona pellucida and sperm penetration into oocyte result in fertilization failure. So major cause of fertilization failure is of sperm origin than oocyte.
(b) Less spermatozoa bind to unfertilized oocytes from IVF than freshly collected oocytes. Preovulatory oocytes bind to higher number of sperm than oocytes harvested from immature follicles.
(c) Low sperm binding in vitrified oocytes.

20.6 Heterologous Sperm-Zona Pellucida Assay

This is another in vitro method of evaluation of sperm fertilizing ability where the sources of oocytes are a constraint; for example, in equines. In this method, the zona binding capacity of spermatozoa of one animal can be tested using oocytes of an animal of a related species [35].

Preparation of Porcine Oocytes

(a) Immature cumulus oocyte complexes (COCs) harvested from ovaries of slaughtered gilts can be used in the test.
(b) Transport porcine ovaries to laboratory in NSS fortified with antibiotic (gentamicin at 50 μg/ml) within 2 h of slaughter.
(c) Aspirate the follicular fluid along with oocytes using 18 gauge needle attached to a 5 ml syringe. Isolate good-quality oocytes and put them in droplets of OCM.
(d) Wash the oocytes with DPBS once and subsequently with maturation medium.
(e) Incubate the oocytes in a 50 μl droplet of maturation medium in a CO_2 incubator in an atmosphere of 5% CO_2 and 95% humidity at 38.5 °C.
(f) Denude the mature COCs mechanically by washing vigorously with modified Tris-buffered medium.

Preparation of Equine Spermatozoa

(a) Thaw the frozen equine spermatozoa at 37 °C for 30 sec.
(b) Wash the spermatozoa with Beltsville thawing solution by centrifuging at 600 g for 10 min.
(c) Collect the motile sperms by centrifuging the sperm pellet on a Percoll discontinuous gradient at 700 g for 30 min.
(d) Collect the sperm at the bottom of the fraction, and wash with mTBM by centrifuging at 100 g for 10 min.
(e) Suspend the sperm pellet in mTBM with a final concentration of 10×10^6/ml.
(f) Evaluate the sperm motility in the suspension.

Procedure

(a) Co-incubate denuded porcine oocytes with the sperm suspension (10×10^6/ml) in mTBM in a CO_2 incubator at 38.5 °C, 5% CO_2 and 100% humidity for 30 min.
(b) After incubation, fix the sperm-oocyte complex with 1% paraformaldehyde in PBS for 10 min at room temperature.
(c) Count the bound spermatozoa to oocyte with the help of a microscope.

Calculations

$$\text{Zona binding Index} = \frac{\text{No. of sperm bound to the test group oocytes}}{\text{No. of sperm bound to the control group oocytes}}$$

Calculate the index for each pair of test and control group of oocytes. Compare one or two test samples with the control semen sample using oocytes obtained from the same batch of slaughterhouse ovaries.

Critical Parameters

Related species should be considered where sperm of one species can bind to the oocytes of another species.

20.6.1 Background Information

Various experiments suggested that zona pellucida selectively bind the sperms (Fig. 20.4).

Equine zona binds equally with porcine spermatozoa, while fewer equine spermatozoa can bind to porcine zona pellucida. Human ZP binds to sperm of mouse, but reverse is not true [36]. Spermatozoa of rats show higher tendency to bind with ZP of mouse oocyte, but less number of

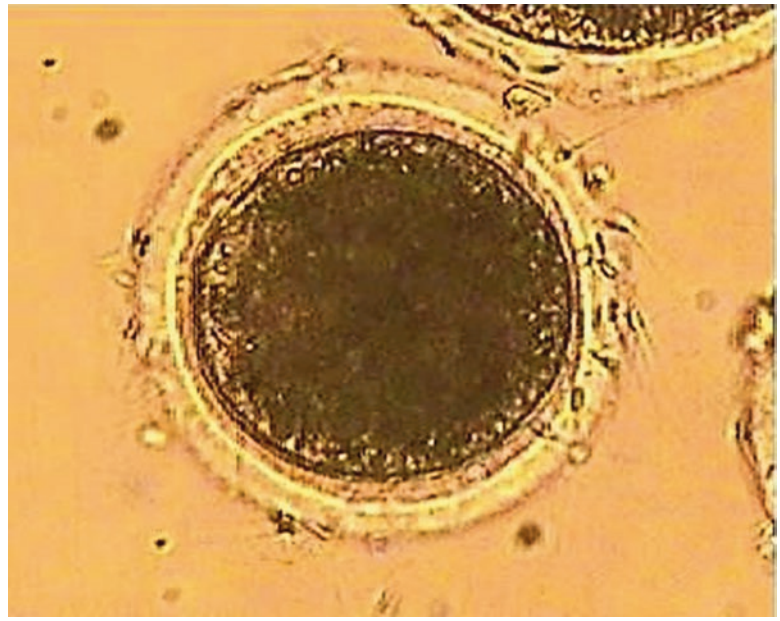

Fig. 20.4 Micrograph shows binding of capacitated cattle bull spermatozoa with buffalo oocyte (heterologous zona binding assay)

mouse spermatozoa bind to rat ZP. Equine spermatozoa show zona binding activity with bovine oocytes [37]. These findings suggest that ZP of oocytes of some animals support the heterologous sperm binding, whereas that of others does not permit for the same.

While interpreting results, an investigator may decide to calculate either the total number of spermatozoa bound to each oocyte (percent zona binding, Fig. 20.4) or zona binding index as explained above.

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