Assiut Vet. Med. J. Vol. 45 No. 89, April 2001

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COMPARISON OF NUMBER AND QUALITY OF OOCYTES IN THE EGYPTIAN BUFFALOES (*BUBALUS BUBALIS*), COWS (*BOS TAURUS*) AND CAMELS (*CAMELUS DROMEDARIUS*)

(With 2 Tables and 6 Figures)

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(Received at 29/3/2001)

مقارنة عدد وخصائص البويضات في إناث الجاموس والأبقار والنوق المصرية

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أجري هذا البحث بهدف معرفة مدى مقدرة إناث الجاموس والأبقار والنوق المصرية على إنتاج عدد من البويضات المناسبة لعملية الإخصاب خارج الرحم. أجري البحث على عدد ٣٢ مبيض من الجاموس و ١٢ من الأبقار و ١٢ من النوق تم الحصول عليها من مجازر محلية. نقلت المبايض إلى المعمل وتم إحصاء عدد الحويصلات الموجوده على سطح المبايض والتي يتراوح حجمها بين ٣-٨ مم. تم سحب مكونات هذه الحويصلات بواسطة إبرة ذات قطر داخلي G19 ومتصلة بمحقن. تم جمع السائل الجريبي في أنابيب زجاجية وتركه لمدة ١٥ دقيقة حتى تترسب البويضات. تم سحب الراسب من قاع الأنبوبة بواسطة ماصة وفحصت ميكروسكوبيا بعد وضعها في أطباق بترى قطر ٣,٥ سم وذلك بدرجة تكبير ١٠٠×. تشير النتائج المتحصل عليها أن متوسط عدد الحويصلات لكل مبيض والتي يتراوح حجمها بين ٣-٨ مم كانت أعلى في الأبقار (١١,١٧) والجمال (١٤.١٧) عنه في الجاموس (٦.٥٦). كما تشير النتائج أن بويضات الجمال ذات الركام البيضي المتراوح بين ٣-٥ طبقة (٦٠%) أعلى من كل من الأبقار (٣١,٤%) والجاموس (٤٣,٧) . وفي نفس الوقت أشارت النتائج أن الأبقار تعطي عدد أكثر من البويضات ذات الركام البيضي أكثر من ٥ طبقات (٣٣,٧%) عن الجمال (٥,٧%) والجاموس(١٤,٩%). كما أظهرت النتائج أن معظم البويضات في الجمال (٩٦,٩%) والأبقار (٩١,٨%) كانت ذات ركام مبيضي متلاصق عنه في الجاموس (٧٧,٤%). كما كانت معظم البيوضات في الجمال ذات سيتوبلازم متجانس (٩٢,٥%) مقارنة بالأبقار (٧٠,٩%) والجاموس (٦٦,٧%). وعليه فإن النتائج تشير إلى أن الأبقار والنوق المصرية قادرة على إنتاج بويضات ذات كفاءة عالية يمكن إستخدامها في عملية الإخصاب المعملي عنه في الجاموس.

SUMMARY

The present study was carried to study the capability of Egyptian buffaloes, cows and camels to produce a reliable number of acceptable oocytes for the in vitro fertilization programs. A total of 32, 12 and 12 ovaries were respectively collected from slaughtered native buffaloes, cows and she-camels. In the laboratory ovarian follicles between 3-8 mm in diameter were examined, aspirated and collected in centrifuge tubes. The recovered oocytes were examined microscopically to state the number of cumulus layers, the quality of cumulus and the quality of the ooplasm. The results revealed that, the average number of aspirated follicles/ovary was higher in camel (14.17) and cows (11.17) than in buffaloes (5.56) ($p< 0.01$) and that the average number of the recovered oocytes/ovary was much higher in cows (7.17) and camels (6.67) than in buffaloes (2.72) ($p< 0.01$). She-camels produced more oocytes with 3-5 cumulus layers (60%) than buffaloes (43.7%) and cows (31.4%) ($p< 0.05$). The majority of oocytes in camels and cows (>90%) showed a compact cumulus in comparison to buffaloes. The ooplasm of the oocytes was more homogenous in camel than cows and buffaloes ($p< 0.05$). In conclusion, the Egyptian native cows and camels produce a higher number and more culturable oocytes than buffaloes.

Key words: *Oocyte, IVF, buffalo, cow, camel*

INTRODUCTION

Since the introduction of embryo transfer (ET) in the field of animal breeding from 1970 onward, it was possible to produce calves of superior genetic quality. One of the large obstacles of ET is the large variability of dams in response to the gonadotropine hormones (Hahn, 1992 and Ali, 2000). To overcome such hindrance of ET, there is an increase demand to collect the oocyte directly from living animals using a transvaginal ultrasonic follicle aspiration technique (Pieterse et al., 1988) or from the ovaries of slaughtered animals (Lu et al., 1987) and cultivating such oocytes in the laboratory before being finally transferred to the recipient animals.

The first requirement of a successful in-vitro fertilization (IVF) is the collection of a good quality oocytes capable of in-vitro maturation, fertilization and cleavage. A good quality oocyte should have a compact multilayered cumulus and homogeneous ooplasm. On the other hand a

bad quality oocyte shows a less compact or expanded cumulus and the ooplasm appear irregular with dark clusters (Deloos et al., 1991).

As there is an increasing interest in many countries in the use of IVF in the production of embryos, the aim of the current study was to investigate the capability of the Egyptian local breeds of buffaloes, cows and camels to produce acceptable oocytes for the IVF-protocol.

MATERIAL and METHODS

The female reproductive tract of native buffaloes, mature cows and she camels were collected from local slaughter house shortly after evisceration. The ovaries (32 buffalo, 12 cows and 12 camel) were dissected free from the tract and the adnexa and transferred in a previously warmed thermos (35°C) within ca. 1h to the laboratory. In the laboratory, the number of the ovarian follicles between 3 and 8 mm in diameter were enumerated and aspirated using a 19G needle attached to a 2 ml syringe.

Aspiration of the follicles was carried out subcortically. This means that the follicle was not punctured directly, but the ovarian tissue near the follicle was penetrated firstly then the follicle itself was punctured from inside (Ali, 2000). More than one follicle could be penetrated in one occasion.

The aspirated follicular fluids were collected in conical glass centrifuge tubes. After about 15 minutes the sediments were aspirated with Pasteur pipette and poured in small petri dishes and examined under 10x objective light microscope for the presence of oocytes.

The recovered oocytes were examined for the following criteria:

1) Number of the cumulus layers:
   i) Denuded oocytes or those with less than 3 layers (Fig. 1, 2)
   ii) Oocytes with 3-5 layers (Fig. 3)
   iii) Oocytes with more than 5 layers (Fig. 4)
2) Quality of the cumulus layer:
   i) Compact, when cumulus cells were tightly adhered to each other (Fig. 4)
   ii) Expanded, when cumulus cells were dispersed from each others (Fig.5).
3) Quality of the cytoplasm:
   i) Homogeneous, when ooplasm had an allover equal darkness (Fig. 1, 3, 4, 5)

ii) Heterogeneous, when small or large light spots are present within the ooplasm (Fig. 2, 6)

The differences between the means were compared statistically by ANVOA-test. The percentages were compared using CHI-Square-test. The difference was considered significant at a probability of $p< 0.05$.

RESULTS

The number of aspirated follicles and the oocytes recovery rate in buffaloes, cows and camels are presented in Table 1. More follicles were aspirated in cows (average of 11.17 ± 7.6 follicles/ovary) and camels (average of 14.17 ± 6.8 follicles/ovary) than in buffaloes (average of 5.56 ± 3.4 follicles/ovary). This differences was statistically significant ($p< 0.01$). Also, higher number of oocytes were recovered from cow ovaries (mean of 7.17 ± 4.7 oocytes/ovary) and camels (mean of 6.67 ± 3.1 oocytes/ovary) than that from buffaloes (2.72 ± 2.3 oocytes/ovary). This difference was also significant ($p< 0.01$). The oocytes recovery rate was not significantly different between cows (64.2%), camels (47.1%) and buffaloes (48.9%).

The quality of the recovered oocytes in buffaloes, cows and camels are presented in table 2. Out of the recovered 87 oocytes in buffaloes 41.4% were denuded or with less than 3 cumulus layers, 43.7% were with 3-5 layers and 14.9% were with more than 5 layers. From the harvested 86 oocytes in cows, 34.9% were denuded or with less than 3 layers, 31.4% with 3-5 layers and 33.7% with more than 5 layers. Out of the obtained 80 oocytes in camels 32.5% were denuded or with less than 3 layers, 60% were with 3-5 layers and 7.5% were with more than 5 layers. As it could be seen, camels produced more oocytes with 3-5 cumulus layers than both cows and buffaloes ($p< 0.05$). At the same time, cows produced more oocytes with more than 5 layers than that of buffaloes and camels ($p< 0.05$).

In buffaloes, 77.4% of the recovered oocytes were with a compact cumulus, while 22.6% were with an expanded one, in comparison to 91.8% compact and 8.2% expanded in cows and 96.9% compact and 3.1% expanded in camels. It is clear that cows and buffaloes produced more compact oocytes than buffaloes ($p< 0.05$).

Out of the recovered oocytes in buffaloes, 66.7% were with a homogeneous ooplasm and 33.3% were with heterogeneous one. In cows the percentages were 70.9% and 29.1% respectively, while the

respective percentages in camels were 92.5% and 7.5%. It could be observed, that the ooplasm of the camel oocytes was in the majority of cases more homogenous than that of buffaloes and cows ($p< 0.05$).

DISCUSSION

Although the IVF-system is well established in cattle, such program is in its initial steps in buffaloes and camels. The first IVF-buffalo calf was born in Japan (Suzuki et al., 1992). A small-scale studies on IVF in the Arabian camel was described by Bou et al. (1993).

In the present study, a higher number of follicles per ovary between 3 and 8 mm in diameter could be aspirated in the Egyptian camels and cows than in the buffaloes. This difference could be due the fact that, the number of primordial follicles and Graafian follicles ≥1 mm in diameter in the ovaries of the buffalo heifers were much lower than in cattle. Samad and Nassari (1979) reported that around 19,000 primordial follicles were available in buffalo ovary which is much lower than 133,000 in cattle at birth (Erickson, 1966). Danell (1987) reported an average of 12,000 primordial follicles in cyclic and 10, 000 in non-cyclic Indian heifers. The number of Graafian follicles >1 mm in cows was about 90 per each pair of ovaries (Rajakoski, 1960), while in buffalo heifers it was about half of that, 46.3 (Danell (1987). Actually, there is no previous study concerning the number of ovarian follicles which could be aspirated in the local breed of cows and camels, and it seems that this is the first report in this concern.

In the current work, the number of the recovered oocytes per ovary was much higher in cows and camels than in buffaloes. In cattle, Katska (1984) reported an average of 10 oocytes/ovary which is slightly higher than 7 oocytes/ovary in the present work. In Indian buffaloes, Sharma and Taneja (2000) reported an average of 2.9 oocytes/ovary which is comparable to 2.7 in the current study. In camels, the number of recovered oocytes (6.7) was much higher than in buffaloes (2.7). On the other hand, it was expected that the number of recovered oocytes should be higher in camel than in cows, because the camel showed slightly higher number of follicles (14.16) than cows (11.16). Actually, the number of recovered oocytes was slightly lower in camel than that of cows (7.2). Part of this explanation may lie in the fact that, the camel ovary seemed as a bunch of grapes with many ovisacs protruding on its surface, which made the ovarian manipulation during follicle aspiration relatively difficult. Also, the ovarian stroma in camels was more firmer

than in cattle, which made its penetration and the subcortical follicle aspiration relatively difficult.

The oocyte recovery rate, that means the number of recovered oocytes per the number of the aspirated follicles, was not different between the three animal species, but it was slightly higher in cows (64.2%) than in camels (47.1%) and buffaloes (48.9%). In fact, the oocytes recovery rate reflect the technique of follicle aspiration, which was equal for all ovaries. The slightly higher recovery rate in cows might be due to the nature of the its ovarian stroma, which was easily to penetrate. KATSKA (1984) recorded a recovery rate of 43% in cattle, which is much lower than the present one.

The number and quality of the cumulus layers depend on the nature of cumulus (healthy or necrotic), diameter of the used needle and the applied pressure during follicle aspiration (Bols et al., 1997). A higher percentage of bovine oocytes showed more than 5 cumulus layers in comparison to camels and buffaloes. Firmer tissue as in case of buffalo and camel ovaries need more pressure which has an adverse effect on the oocyte number.

Cows and camel showed more compact and less expanded cumulus than in buffaloes. Expanded oocytes is routinely excluded from in the IVF-programs.

Of interest is that, the majority of camel oocytes were homogeneous, in comparison to cows and buffaloes. Heterogencity is a signs of follicular degeneration or atresia. Higher level of atresia was recorded in buffaloes than in cattle. About two third of all follicles ≥1 mm in buffalo heifers were atretic (Danell, 1987), in comparison to 50% in cattle (Rajakoski, 1960)

In conclusion, the Egyptian local breed cows and camels produced a higher number and more culturable oocytes than that of buffaloes. In buffaloes, another technique of oocyte recovery might be needed to improve the number and quality of the recovered oocytes. This might need further studies.

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Table 1: Number of aspirated follicles and the oocytes recovery rate in buffaloes, cows and camels

Parameter	Buffaloes N = 32	Cows N = 12	Camels N = 12
Total number of aspirated follicles	178	134	170
Number of aspirated follicles/ovary (mean + s.d.)	5.56 ± 3.4[a]	11.17 ± 7.6[b]	14.17 ± 6.8[b]
Total number or recovered oocytes	87	86	80
Number of recovered oocytes/ovary (mean ± s.d.)	2.72 ± 2.3[a]	7.17 ± 4.7[b]	6.67 ± 3.1[b]
Oocyte recovery rate (%)*	48.9[a]	64.2[a]	47.1[a]

Values with different superscript letters on the same row differ significantly
N: Number of ovaries
**Oocyte recovery rate = number of recovered oocytes x 100 / number of aspirated follicles*

Table 2: Quality of the recovered oocytes in buffaloes, cows and camels

Quality of oocytes	Buffaloes n = 87	Cows n = 86	Camels n = 80
I. According to the number of cumulus layers			
Denuded oocytes or those < 3 layers- (%)	41.4[a]	34.9[a]	32.5[a]
Oocytes with 3-5 layers (%)	43.7[a]	31.4[a]	60.0[b]
Oocytes with 6-10 layers (%)	14.9[a]	33.7[b]	7.5[c]
II. According to the quality of the cumulus layer			
Compact oocytes (%)	77.4[a]	91.8[b]	96.9[b]
Expanded oocytes (%)	22.6[a]	8.2[b]	3.1[b]
III. According to the quality of the ooplasm			
Homogenous oocytes (%)	66.7[a]	70.9[a]	92.5[b]
Heterogeneous oocytes (%)	33.3[a]	29.1[a]	7.5[b]

Values with different superscript litters on the same row differ significantly
n: number of the recovered oocytes

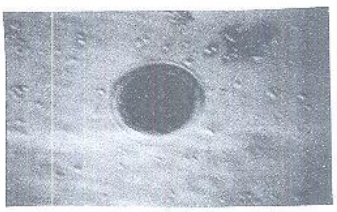

Fig. 1: Denuded buffalo oocyte with homogeneous ooplasm, 100x

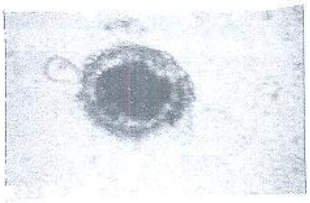

Fig. 2: Buffalo oocyte with < 3 cumulus layers and heterogeneous ooplasm, 100x

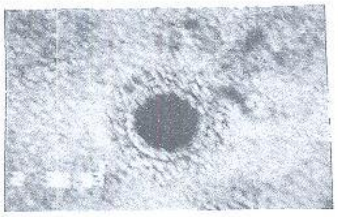

Fig. 3: Camel oocyte with 3 compact cumulus layers and homogeneous ooplasm, 100x

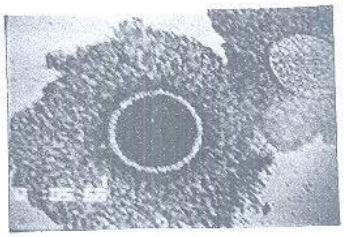

Fig. 4: Cow oocyte with >5 compact cumulus layers and homogeneous ooplasm, 100x

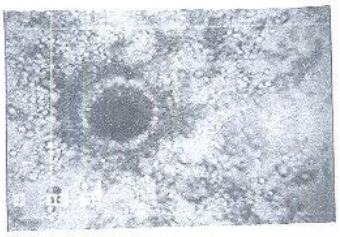

Fig. 5: Buffalo oocyte with expanded cumulus layers and homogeneous ooplasm, 100x

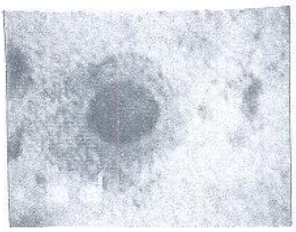

Fig. 6: Cow oocyte with 5 compact cumulus layers and heterogeneous ooplasm, 100x